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THE TAXONOMY AND DEVELOPMENT  
OF *APIOSPORINA COLLINSII* (SCHW.) VON HÖHNEL

by

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A THESIS

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "THE TAXONOMY AND DEVELOPMENT OF *APIOSPORINA COLLINSII* (SCHW.) VON HÖHNEL", submitted by Archibald William Lewis Stewart, in partial fulfilment of the requirements for the degree of Master of Science.





## ABSTRACT

The ontogeny of *Apiosporina collinsii* (Schw.) von Höhnelt was investigated. The occurrence of an ascostromatic ascocarp is shown and the development of pseudoparaphyses in the centrum is discussed. The mode of development of the ascocarp of this species is typical of the *Pleospora*-type and the production of ascogenous hyphae is similar to that of the *Lophodermium juniperinum* - Type I. The description of *Apiosporina collinsii* is emended and the original generic description of *Apiosporina* revised to include the species *Apiosporina morbosa* (Schw.) v. Arx. The effects of these parasites on their hosts are discussed and their geographical distribution is reported.

The classification of the Pyrenomycetes is reviewed to show the basis for the separation of the ascostromatic ascomycetes from this group and the formation of the subclass Loculoascomycetes for these fungi.

The generic limits of *Apiosporina* are discussed and the morphological features aiding in its classification are evaluated. The two species, *Apiosporina collinsii* and *Apiosporina morbosa*, are classified in the subclass Loculoascomycetes, order Pleosporales and family Venturiaceae.





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## INTRODUCTION

*Apiosporina collinsii* (Schw.) von Höhnelt is a parasitic fungus attacking species of *Amelanchier* and causing the formation of witches' brooms on the host. The disease is encountered frequently in the Edmonton area on *Amelanchier alnifolia* Nutt., commonly known as the Saskatoon.

This fungus was previously considered a member of the vast group of Ascomycetes called the Pyrenomycetes. Within this group, however, *Apiosporina collinsii* has never been classified with ease. Developmental studies on this species were conducted by Sartoris and Kauffman (1925) and Sprague and Heald (1927). The former authors placed this fungus in the Sphaeriaceae saying, "that its connection with the Sphaeriaceae is far closer than with any other group shown by its characters other than the lack of an ostiolate perithecium", and ended their discussion by stating that its position in this family was "fairly assured". Sprague and Heald (1927) also placed this species in the Sphaeriaceae "as its perithecial wall is distinct from the subiculum". They commented that, "the fact that this fungus has been listed in the Perisporiales, Dothi-deales and Sphaeriales at different times emphasizes the need for a more careful study of the Pyrenomycetes". Since the time of their work, the fungus has also been considered a member of the Pseudosphaeriales (Miller 1938) and the





Erysiphales (Ainsworth and Bisby 1954).

The lack of a suitable resting place for *Apiosporina collinsii* was due to the lack of knowledge of the development of the species making up the Pyrenomycetes. Luttrell (1951) began to revise this group on the basis of developmental studies available at that time, and has since offered (Luttrell 1955, 1965) a revised scheme which will help to clear up many of the problems of classification of the pyrenomycetous fungi.

Luttrell (1951) states that, "because of the odd account of its development given by Sartoris and Kauffman in their text, however, *Apiosporina collinsii* must be re-investigated before it can be referred to any recognized developmental type".

With this in mind, a detailed study of this organism was conducted involving developmental studies of the life-history of the fungus from the vegetative mycelium in the host to the release of mature ascospores. In addition, investigations of the effects of the fungus on the host tissues were extended beyond those reported by Sartoris and Kauffman (1925) and Sprague and Heald (1927). On the basis of the information obtained from these studies, a classification of *Apiosporina collinsii* is proposed and the generic limits of *Apiosporina* are discussed.

During the course of the investigation, a report (Farr 1963) of two other species, *Apiosporina morbosa* (Schw.) v. Arx and *Apiosporina harunganae* Hansf. was



discovered. The former proved to be *Dibotryon morbosum* re-named by von Arx (1954) and the latter was a new species described by Hansford (1947). Herbarium specimens of *Apiosporina morbosum* were obtained and examined for developmental stages, effects on the host, and distribution of the fungus. The results were compared with those from *Apiosporina collinsii* to determine the similarities and differences occurring between the two species in order to ascertain whether or not they were members of the same genus. Specimens of *Apiosporina harunganae* were not available for examination, nor has there been any work published on this species since its original description, to the best of my knowledge.

In this thesis, a review of the history of the taxonomy of the fungi previously known as the Pyrenomycetes is presented. The classification of these fungi has been undergoing constant change ever since the group was formed by Fries in 1823 and the account provides the background needed to evaluate the decisions made in classifying the fungi of this study.

The findings of this study concerning *Apiosporina collinsii* and *Apiosporina morbosum* are presented in the following manner. Observations on the life histories and pathogenicity studies of these fungi are presented in the observations section under the headings:

1. The Ontogeny of the Fungus
2. The Effects on the Host



Information on the geographical distribution of these species is given in the section entitled "Geographical Distribution".

The discussion is divided into two sections. The first section deals with the life history of *Apiosporina collinsii* as this species was the one of major interest in this study. The second part of the discussion is concerned with the taxonomy of the genus *Apiosporina*. Here the description of the species *Apiosporina collinsii* is emended to include new information from this study and the generic limits of the genus are set by the revision of the original description of *Apiosporina* by von Höhnelt (1910). This section also includes the classification of the genus and a discussion of the features taken into account in arriving at this classification.





## LITERATURE REVIEW

### A. Taxonomy of the Pyrenomycetes

The vast group of ascomycete fungi known as the Pyrenomycetes was formed by Fries in 1823 as an order of the class Basidiomycetes. Since this time numerous workers have reinvestigated the species comprising the group in order to define the orders and families of the Pyrenomycetes more precisely.

The earliest divisions within the Pyrenomycetes were based on characters such as color and consistency of perithecial walls and stroma, presence or absence of a stroma and the position of the ascocarps with reference to their substrate. On the basis of such characteristics, Lindau, in Engler and Prantl, established the order Pyrenomycetineae in 1897 to include fungi of the class Ascomycetes having closed ascocarps which opened by disintegration or by means of an apical ostiole and contained a "nucleus" composed of asci which arose as a single layer or cluster in the base of the ascocarp and usually contained sterile threads or paraphyses. The order was divided as follows:

#### Pyrenomycetineae

Suborder I	Perisporiales
Family 1	Erysiphaceae
2	Perisporiaceae
3	Microthyriaceae



Suborder II      Hypocreales

Family 1      Hypocreaceae

- |           |   |               |
|-----------|---|---------------|
| Subfamily | 1 | Hyponectrieae |
|           | 2 | Hypomyceteae  |
|           | 3 | Melanosporeae |
|           | 4 | Nectrieae     |
|           | 5 | Hypocreeae    |
|           | 6 | Clavicipiteae |

Suborder III      Dothideales

Family 1      Dothideaceae

Suborder IV      Sphaeriales

- |        |    |                    |
|--------|----|--------------------|
| Family | 1  | Chaetomiaceae      |
|        | 2  | Sordariaceae       |
|        | 3  | Sphaeriaceae       |
|        | 4  | Ceratostomataceae  |
|        | 5  | Cucurbitariaceae   |
|        | 6  | Coryneliaceae      |
|        | 7  | Amphisphaeriaceae  |
|        | 8  | Lophiostomataceae  |
|        | 9  | Mycosphaerellaceae |
|        | 10 | Pleosporaceae      |
|        | 11 | Massariaceae       |
|        | 12 | Gnomoniaceae       |
|        | 13 | Clypeosphaeriaceae |
|        | 14 | Valsaceae          |
|        | 15 | Melanconidaceae    |
|        | 16 | Diatrypaeae        |
|        | 17 | Melogrammataceae   |
|        | 18 | Xylariaceae        |

Classification systems such as this, based on gross morphology lead to artificial groupings of species and do not indicate natural relationships between organisms. Nor do such schemes lend themselves to the determination of phylogeny within these groups. According to Luttrell (1951) the general outline of Lindau's system was usually followed until his time of writing.



In the numerous papers published in the past sixty years on the development and cytology of individual species of the group referred to as Pyrenomycetes, there has been a tendency to recognize a distinct series of Pyrenomycetes which are ascostromatic in nature. Previously, the group was characterized as having perithecial ascocarps which were hollow, flask-shaped structures that opened by an apical ostiole and were lined with a single layer of asci interspersed with paraphyses. These perithecia could be separate structures or joined by a stroma. Perithecia in the stromal forms could be either on the surface of the stroma or embedded in the pseudoparenchymatous tissue to varying degrees. It was soon recognized that all species did not have true perithecia. Ascomycetes like *Dothidea* were discovered to have asci in perithecia-like cavities or locules in a large stroma in which a differentiated perithecial wall was lacking. The discovery of other Ascomycetes like *Dothidea* led to the establishment of the family Dothideaceae by Nitschke and Fuckel in 1869. This family was raised to ordinal rank by Lindau in 1897. At the time this order was formed it was composed of heterogeneous species, some having true perithecia which only superficially resembled the *Dothideae*-type and some which were simple ascostromatic forms containing asci in a single locule which closely resembled the true perithecial forms of the Sphaeriales.

Von Höhnelt, according to Luttrell (1951), segregated the ascostromatic forms, and, in 1907, established





the family Pseudosphaeriaceae in the Dothideales for genera in which the ascocarp is a single perithecium-like stroma containing asci separated by strands of sterile tissue or interthecial threads. Von Höhnel believed that these interthecial threads resulted from the compression of interascal stromal tissue when the asci expanded.

In 1909, von Höhnel expanded the concept of the Dothideales to include the Pseudosphaeriaceae and the Myriangiaceae. The latter was a family of Ascomycetes which produced asci in monoascus locules at various levels of a large stroma.

Von Höhnel's classification of the Dothideales (in Luttrell, 1951) was as follows:

#### Dothideales

Family 1	Dothideaceae	- polyascus locules in a stroma
Family 2	Pseudosphaeriaceae	- monoascus locules in a perithecial stroma
Family 3	Myriangiaceae	- monoascus locules in non-perithecial stromata

Theissen and Sydow (1918) erected the Dothidiineae to contain the Myriangiales, Dothideales and Pseudosphaeriales as an extension of von Höhnel's concept. However,





according to Luttrell (1965), von Höhnelt opposed this extension.

The orders of Theissen and Sydow were characterized as follows:

- |                   |   |
|-------------------|---|
| Myriangiales      | - species with broad, cushion<br>-shaped, Discomycete-like<br>ascostromata              |
| Dothideales       | - compound forms with perithecia<br>-like locules in a pulvinate<br>stroma              |
| Pseudosphaeriales | - simple forms with perithecia<br>-like stromata, containing<br>perithecia-like locules |

The final classification of the Dothidiineae of Theissen and Sydow (1918) was as follows:

- |          |                    |
|----------|--------------------|
| Order I  | Myriangiales       |
| Family 1 | Elsinoeae          |
| 2        | Plectodiscelleae   |
| 3        | Myxomyriangiaceae  |
| 4        | Myriangiaceae      |
| 5        | Saccardiaceae      |
| 6        | Dothioraceae       |
| Order II | Pseudosphaeriales  |
| Family 1 | Epipolaeaceae      |
| 2        | Parodiellaceae     |
| 3        | Botryosphaeriaceae |
| 4        | Cucurbitariaceae   |
| 5        | Pleosporaceae      |



Family 6	Sphaerellaceae
7	Pseudosphaeriaceae
8	Lichenicolae

### Order III      Dothideales

Family 1	Coccoideaceae
2	Leveilleleae
3	Dothideaceae

The family Dothioraceae contained species with interthecial tissue compressed into paraphysoidal threads between the asci and was considered transitional to the Pseudosphaeriales in which the ascostromata became perithecium-like.

Luttrell (1951) stated that the concept of the Pseudosphaeriales as delimited by Theissen and Sydow (1918) has never been accepted. He said that Petrak in 1923, totally rejected the order saying it was composed of heterogeneous forms and advocated its dissolution. Petrak considered the Pseudosphaeriaceae in the sense of von Höhnelt to be primitive forms of the Pleosporaceae and placed them in the Pleosporaceae (Sphaeriales) which he divided into two subfamilies; the Pseudosphaerieae and the Pleosporeae.

Petrak's classification united parts of the ascostromatic forms with parts of the perithecial forms in the same developmental series. Thus he opposed the view that they constituted two entirely distinct series. According to Luttrell, Gäumann in 1926 accepted most of Petrak's



ideas, but also considered the Pseudosphaeriaceae as transitional from the Myriangiales to the Sphaeriales and retained the family in the former order.

Luttrell noted that Arnaud in 1925 agreed with Petrak's scheme, and said that since there was no definite character available which could be used to distinguish simple Sphaeriales from uniloculate ascostromatic forms, the Pseudosphaeriales should be retained in the Sphaeriales pending further study.

Miller (1928) renewed the idea that the ascostromatic Pyrenomycetes represented a distinct series of the Ascomycetes. He pointed out the falsity of the conception of Petrak and Gäumann that perithecia could be derived from ascostromata. He emphasized that perithecial walls were specialized structures which could not be considered homologous with stromal tissue, and that paraphyses could not be derived from compressed intertheccial stromal tissue. Miller also believed that true perithecia opened by means of schizogenous ostioles, while stromal forms opened by means of lysigenous aperiphysate pores. Emphasis on this character as a criterion for determining true perithecia is diminishing as more recent developmental studies have shown that a number of ascostromatic species possess periphysate ostioles.

Miller separated the ascostromatic Pyrenomycetes from true Pyrenomycetes on the basis of ascocarp form. The group considered by him to be true Pyrenomycetes pro-







duced true perithecia and included only the Sphaeriales, Hypocreales and the Erysiphaceae. He felt that this group was quite distinct from the ascostromatic forms placed in the Myriangiales, Pseudosphaeriales, Dothideales, Perisporiaceae and Coryneliaceae. At this time he felt that the Dothideales should include the Pseudosphaeriales.

Clements and Shear (1931) disagreed with the validity of the concept of the Pseudosphaeriaceae, and placed all ascostromatic forms in one order, the Dothideales. They divided it into three families; the Dothideaceae, the Myriangiaceae and the Mycoporaceae. The majority of genera which were considered by others to belong to the Pseudosphaeriales were retained in the Sphaeriales and Perisporiales.

In 1932, Nannfeldt, a student of the Ascomycetes, proposed a system of classification which divided the Euascomycetes into three series; the Plectascales, the Ascohymeniales and the Ascoloculares. Pyrenomycetes with true perithecia were placed in the Ascohymeniales, while ascostromatic forms composed the Ascoloculares. Nannfeldt's Ascoloculares consisted of four orders; the Myriangiales, Pseudosphaeriales, Hemisphaeriales and Trichothyriales. The Pseudosphaeriales was a large and poorly defined group and included the family Pleosporaceae which is of particular interest in this study.



Miller (1938) redefined the Dothideales and Pseudosphaeriales on the basis of the internal structure of the locule rather than on the number of locules in a stroma as used by Thiessen and Sydow (1918). He stressed the point that the interthecial threads or paraphysoids of the Pseudosphaeriales were distinct hyphae, originating from the apex of the locule. These hyphae grew downward and became attached to the bottom of the locule. This feature becomes increasingly important in more recent classification schemes. Miller placed all ascostromatic forms with this feature into the Pseudosphaeriales. His Dothideales contained forms lacking paraphysoids and in which the asci arose in fascicles in pseudoparenchymatous locules.

According to Luttrell (1951), Gäumann in 1940 placed all ascostromatic forms in a single order, the Pseudosphaeriales. He divided this order into the Dothioraceae, Pseudosphaeriaceae, Mycosphaerellaceae and Dothideaceae.

Martin (1945) proposed four orders for all ascostromatic Ascomycetes. These were the Myriangiales, Dothideales, Microthyriales, and Meliolales. In the Dothideales he included the ascostromatic forms with perithecium-like ascocarps and he placed the family Pseudosphaeriaceae in this order.

Miller's (1949) revision of the classification of the Ascomycetes is based primarily on characters of the ascus correlated with ascocarp centrum characteristics.



He believed this system provided a more natural scheme of classification than Nannfeldt's. Miller's studies were concerned primarily with the Pyrenomycetes, especially those previously placed in the Sphaeriales, Dothideales and Hypocreales. The key provided by Miller to orders and families of the fungi concerned was based on ontogeny, characteristics of the mature apothecial or perithecial centra and morphological features of the ascus and ascospores. Characters such as position of the ascocarps in relation to substrate and color or extent of stroma were not considered fundamental characters and were ignored. He retained the old Ascomycete subclasses of the Euascomycetes; the Plectomycetes, Pyrenomycetes and Discomycetes. The subclass Pyrenomycetes included all fungi with asci borne in parallel series in closed fruiting bodies which opened by means of a definite pore or slit. He divided the Pyrenomycetes into orders as follows:

Subclass		Pyrenomycetes
Order	1	Laboulbeniales
	2	Sphaeriales
	3	Hypocreales
	4	Dothideales
	5	Pseudosphaeriales
	6	Micothyriales
	7	Lophiostromatales
	8	Hysteriales

The orders of particular interest here are the Sphaeriales, Dothideales, Pseudosphaeriales and Micothyri-





ales. The Dothideales were characterized as those fungi having asci arising in fascicles within locules formed by the dissolution of pseudoparenchymatous stromal tissue whether uni- or multiloculate. In the Pseudosphaeriales were placed fungi in which the asci arose from a concave layer across the bases and sides of the locule and were interspersed with pseudoparaphyses. The majority of the ascostromatic forms were placed in the Pseudosphaeriales. Fungi with similar centrum structure, but with dimidiate, shield-shaped stromata were considered to belong to the Microthyriales. The Sphaeriales contained species with asci interspersed with apically free paraphyses and globose perithecia with ostioles formed by the extension of wall tissue and lined with periphyses.

Miller stated that species producing single perithecia could be separated from those having uniloculate stromata on the basis of characteristics of the ascus. Those having apically thickened asci with pores belonged to the Sphaeriales, while those with asci lacking pores and whose outer wall split to eject the spores were loculate forms. He further pointed out that asci in the Sphaeriales gave a blue reaction with iodine while those of others did not.

According to Luttrell (1951), Gäumann recognized the ascostromatic forms as a distinct series and used Nannfeldt's divisions of the Euascomycetes. He included the Perisporiales, Myriangiales, Pseudosphaeriales and





Hemisphaeriales in the Ascoloculares.

Bessey (1950) divided the ascostromatic Ascomycetes into the Dothideales, Pseudosphaeriales and Hemisphaeriales. He separated the Dothideales and Pseudosphaeriales on the number of locules according to the method employed by Theissen and Sydow in 1918 and ignored centrum structure as emphasized by Miller in 1938.

The result of Luttrell's study (1951) was the proposal to divide the Euascomycetes into two major series based primarily on the occurrence of bitunicate or unitunicate asci in the ascocarps. The two series were named the Unitunicatae and the Bitunicatae. Luttrell found that when this character was given primary importance, those species with bitunicate asci "almost invariably" lacked true perithecial walls. He felt that separation of the Pyrenomycetes on this basis would yield two distinct series which were of the same order as those based on ascostromatic and perithecial forms.

Luttrell divided his series Bitunicatae into five orders. These were the Myriangiales, Dothideales, Pseudosphaeriales, Hysteriales and Trichothyriales. He considered the Bitunicatae to be equal to Nannfeldt's Ascoloculares. Luttrell's handling of the Pseudosphaeriales is of particular interest to this thesis. The Unitunicatae was divided into the subseries Plectomycetes, Pyrenomycetes, Laboulbeniomycetes and Discomycetes. In this way the Pyrenomycetes became limited to those Ascomycetes with unitunicate



asci and true perithecia. Luttrell defined a true perithecium as, a closed, spherical or flask-shaped ascocarp consisting of a wall which arises as a result of a stimulus derived from the presence of an ascogonium and a centrum in which the asci are formed. He further divided the Unitunicatae and the Bitunicatae into orders and families on the basis of other ascocal characters and on features of the centrum. From his reviews of centrum structure, he recognized eight primary types of centrum development. The series Bitunicatae and the centrum development types associated with fungi placed in this series are of particular interest in connection with this study.

Luttrell (1955) believed that the ascostromatic forms represented a distinct line of development within the Ascomycetes that was separate from the line of development of true Pyrenomycetes. He proposed that the ascostromatic series be given definite taxonomic rank and he placed this series in a new subclass called the Loculoascomycetes. The name of the subclass was derived from Nannfeldt's *Ascoloculares* by transposing the syllables to give a name corresponding in form to the other subclasses of the Ascomycetes. Luttrell suggested that the species with bitunicate asci constituted a separate monophyletic line and considered the bitunicate ascus as the prime character of his new subclass. He ranked the ascostromatic nature of the ascocarps as a secondary correlated character.

At this time, Luttrell divided the Loculoasco-





mycetes into six orders. These were the Myriangiales, Dothideales, Trichothyriales, Pleosporales, Hysteriales and Microthyriales. The order Pleosporales was proposed to replace the order Pseudosphaeriales as outlined by Miller. His reason for doing this was that the order Pseudosphaeriales had been used in so many different senses in the past that it was a source of confusion. Under his proposed scheme of classification, the family Pseudosphaeriaceae with its type genus *Pseudosphaeria* was transferred to the Dothideales. This made the Pseudosphaeriales a synonym of the Dothideales.

Müller and von Arx (1962) disagreed with Luttrell's system of classifying the ascostromatic forms on the basis of centrum characters. They felt that between the pseudoparenchymatous centrum and the pseudoparaphysoid type, all possible transitional types occurred. It was their belief that Luttrell provided a static system and that natural groups like the Mycosphaerellaceae and Venturiaceae would be separated. Furthermore the groups could not be placed in either the Dothideales or Pleosporiales. It is at this point that the differences between the classification of Luttrell and that of Müller and von Arx are most evident. According to Luttrell's system, based on centrum structure, these two families would not be placed together as the ascostromata of the Mycosphaerellaceae are aparaphysate while those of the Venturiaceae are pseudoparaphysate.

Müller and von Arx retained the order Pseudo-





sphaeriales for asculocular, bitunicate Pyrenomycetes. They described the order as a group of Asculoascomycetes and Pyrenomycetes with fruit bodies that opened by means of a pore or canal. Their remarks may be translated as follows: "in higher forms the canal was frequently delimited earlier, but was closed by a group of smaller celled pseudo-parenchymatous tissue or was clad with periphyses and was later, often clogged by the ends of paraphysoids". They considered that a typical member of the Pseudosphaeriales contained asci which were cylindrical, ellipsoid, egg or sac-shaped. The lower third of the ascus could be wider than the top and the asci could be short stalked.

Müller and von Arx divided the Pyrenomycetes having two-celled spores into five orders. These were the Dothiorales, Pseudosphaeriales, Phacidiales, Sphaeriales and Plectascales. The order Pseudosphaeriales was divided into twelve families as follows:

Order: Pseudosphaeriales

Family	1	Pleosporaceae
	2	Lophiostomaceae
	3	Mesnieraceae
	4	Sporormiaceae
	5	Mycosphaerellaceae
	6	Venturiaceae
	7	Dimeriaceae
	8	Capnodiaceae
	9	Microthyriaceae
	10	Micropeltaceae
	11	Chaetothyriaceae
	12	Trichothyriaceae







unaltered stromal tissue.

2. *Pseudosphaeria*-type - asci arise in monascus  
locules, but are so crowded  
that the interthecial  
tissue is compressed into  
strands or disintegrates.
3. *Dothidea*-type - asci push up in compact  
groups into the disinte-  
grating stromal tissue.
4. *Pleospora*-type - locule formed by growth of  
pseudoparaphyses among which  
the asci develop.

The *Pleospora*-type is of main interest in this thesis.

Luttrell's (1955) system of classification of the  
Loculoascomycetes is outlined as follows:

Subclass III		Loculoascomycetes	
Order I		Myriangiales	
Family	1	Atichiaceae	
	2	Myriangiaceae	
	3	Saccardiaceae	
	4	Cryptotheciaceae	
	5	Stephanothecaceae	
Order II		Dothideales	
Family	1	Pseudosphaeriaceae	
	2	Dothioraceae	
	3	Dothideaceae	
	4	Capnodiaceae	





Order III	Trichothyriales
Family 1	Trichothyriaceae
Order IV	Pleosporales
Family 1	Pleosporaceae
2	Lophiostomataceae
3	Venturiaceae
4	Botryosphaeriaceae
5	Mesnieraceae
6	Didymosphaeriaceae
7	Herpotrichiellaceae
Order V	Hysteriales
Family 1	Hysteriaceae
2	Opegraphaceae
3	Arthoniaceae
4	Rocellaceae
Order VI	Microthyriales
Family 1	Microthyriaceae
2	Micropeltaceae

Luttrell revised this classification in 1965 retaining the orders Myriangiales, Pleosporales, Hysteriales and Dothideales and introducing two new orders; the Capnodi-ales and the Hemisphaeriales. The Capnodiales was established for fungi having small, spherical, uniloculate ascocarps opening by a pore or dissolution of the peripheral tissue. The internal structure of the ascocarps was of the *Pseudosphaeria* or *Dothidea*-type with interthecial tissue remaining as strands or disintegrated. The fungi comprising this order were types which were entirely superficial or penetrated the host with haustoria or an "innate" mycelium.





The Hemisphaeriales included the families Trichothyriaceae, Microthyriaceae and Micropeltaceae of his previous (1955) system.

In 1965 Luttrell listed only three families for the order Pleosporales as opposed to seven in 1955. These were the Pleosporaceae, Lophiostomataceae and Sporomiaceae. He remarked that the Pleosporaceae "is the largest group in the Loculoascomycetes and must be divided eventually into a number of families, but the lines of such a division are barely emerging".

## B. *Apiosporina collinsii* (Schw.) von Höhnelt

### 1. Developmental Studies

Developmental studies of *Apiosporina collinsii* were conducted by Sartoris and Kauffman (1925) and Sprague and Heald (1927).

Sartoris and Kauffman give the following account of the development of *Apiosporina collinsii*. The mycelium appears on the under surface of newly emerged *Amelanchier* leaves during the latter part of March or early April. It is suggested by them that the mycelium arises from ascospores present on old leaves attached to the twigs of the witches' broom. Conidiophores develop from the mycelium which is dark brown, appearing black in mass, and superficial until the perithecia form. The conidiophores are



erect, composed of two or three cells and bear unicellular conidia which are uninucleate and lemon-shaped. The abundant conidia give a greenish tinge to the mycelium.

When the perithecia develop, the hyphae of the mycelium become intercellular. These intercellular hyphae have thin, hyaline walls and are full of a granular material. The development of the perithecium begins from a coil of mycelium composed of two slightly differentiated hyphae surrounded by closely packed, vegetative hyphae which will form the perithecial walls. The central cells divide to form many uninucleate cells. When the perithecial wall is completely formed a fan-shaped group of hyphae is sent out from the central coil. These hyphae grow until they reach the top of the perithecium. The asci develop within the fan-shaped group of hyphae in an intercalary fashion and when the asci are mature, the hyphal tips disintegrate. The mature asci contained eight, two-celled ascospores, each having a large upper cell and a much smaller lower cell. Sartoris and Kauffman report that by the end of August, the perithecia are so numerous that the under surface of the infected leaves is covered by a black stroma-like layer mixed with perithecia.

Flask-shaped pycnidia with pycnidiospores are said to occur on the upper surface of infected leaves but these authors do not suggest a function for this stage.

Sprague and Heald's account of the development of the fungus states that the olive-brown conidiophores develop





from a stromatic layer on the underside of the leaves. This layer could be traced through the leaf guard cells to the leaf exterior. The conidiophore is a simple or branched, several-celled structure which gives rise to numerous olive-brown, citron-shaped, one-celled spores from terminal or lateral positions.

The perithecia are described as small, globose, apparently ostiolate, slightly hairy structures seated side by side in the subiculum which covers the lower leaf surface. The description of the ascospores agreed with that of Sartoris and Kauffman. Sprague and Heald were in doubt as to the mode of dissemination of the ascospores, but believed infection occurred through cracks in twig forks.

Müller and von Arx (1962) in their description of *Apiosporina collinsii* stated that fallen leaves of the host become covered on the underside with a crust-forming stroma. This stroma varies in thickness, is anchored to the leaf tissue and forms numerous, crowded protuberances which may fuse with one another laterally while a dematiaceous mycelium develops between them. In their description of the contents of the ascocarps, the asci are said to be surrounded in the beginning by filamentous paraphyses which are joined above and later become mucilaginous. Müller and von Arx (1962) describe the ascospores as hyaline at first, becoming bright brown or yellow at maturity.





## 2. Studies on the Host-Pathogen Relationship

Sartoris and Kauffman (1925) report very little on the host-pathogen interaction of *Apiosporina collinsii* and *Amelanchier canadensis*. They mention the color change, the curling of the host's leaves and the production of witches' brooms, but they did not find the mycelium of the fungus in the stems of the host. It was their opinion that the fungus invaded the host through the lower epidermis of the leaves and grew inward to invade the leaf tissues.

Sprague and Heald (1927) examined the relationship more closely. They described two types of witches' brooms produced by the host: "(a) the compact, short, thickly branched typical form and (b) the looser open type". They noticed the prevalence of the latter in exposed places. The shape of the compact type of broom is accounted for as being caused by the weight of the broom opposing negative geotropism. The reduced angle of branching is also reported.

These authors mention the reduced size of the leaves produced by infected branches and report that the leaves dry up and fall off in early summer. If infected leaves remain on the branch until the following spring, then a second crop of leaves is not produced.

Sprague and Heald discovered the perennial and internal nature of the fungus in the host and reported its presence in the stems, petioles, leaves and flowers. Of its effect on the tissues of the stem, they say that the fungus dissolved the middle lamellae, spreading the cells



apart and that the fungus seemed to cause local disturbances in the cork cambium. The presence of "carbonous" regions in infected stems was reported.

### 3. Review of the Classification of *Apiosporina collinsii*

According to Sartoris and Kaufmann (1925), *Apiosporina collinsii* (Schw.) von Höhnelt was first described by Schweinitz as *Sphaeria collinsii* (Schw.) in 1832. At this time the fungus was assigned to the family Sphaeriaceae.

Thümen transferred this fungus to the genus *Dimerosporium* in 1877.

Peck, in 1878, discovered the fungus on *Amelanchier canadensis* and, under the name *Sphaeria collinsii*, described the microscopic features of the fruiting stage and the effect of the fungus on its host.

According to Sartoris and Kaufmann, Berkeley in 1887 referred two fungi, *Sphaeria russellii* B. & C. and *Sphaeria papillionaceae* B. & C. to the genus *Lasiosphaeria* and indicated that these were synonymous with *Dimerosporium collinsii*.

It was described by Saccardo from the compiled accounts of Schweinitz and Peck as *Dimerosporium collinsii* (Schw.) Thum. in his *Sylloge Fungorum* (1891).

Ellis and Everhart (1892) included it in their treatment of North American Pyrenomycetes in their sub-order Perisporaceae.

Von Höhnelt (1910) examined herbarium specimens of



*Dimerosporium collinsii* and described the perithecia as being ostiolate. On the basis of this feature, he replaced the species in the Sphaeriaceae and named it *Apiosporina collinsii*. At the same time von Höhnelt placed *Plowrightia phyllogena* described by Harkness in his genus and listed it as a synonym of *Apiosporina collinsii*. Von Höhnelt gave the following description for the genus *Apiosporina*:

Fungus almost completely covering the surface, subiculum with branching conidiophores bearing single-celled, colored spores. Perithecia covering the surface, spherical with ostiole and a solid black wall which is sparingly bristly and on a thick turf. Paraphyses filamentous, asci cylindrical, eight-spored. Spores hyaline with a septum towards the base.

Sartoris and Kauffman (1925) and Sprague and Heald (1927) in their work on this fungus agree with von Höhnelt and leave *Apiosporina collinsii* in the Sphaeriaceae.

Miller (1938) lists the genus *Apiosporina* with other genera which he felt should be placed in the new order and family of Thiessen and Sydow (1918), the Pseudosphaeriales and the Pseudosphaeriaceae respectively.

Luttrell (1951) in his treatment of the Pyreno-







mycetes separates species into orders and families on the basis of the type of asci (unitunicate or bitunicate), and the type of centrum development. He places *Apiosporina collinsii* with those species having the *Pleospora*-type of centrum development as described by Brandiff (1936) and Miller (1938). Luttrell suggested that *Apiosporina collinsii* should be reinvestigated before it is placed in a particular group. This author also states that the order Pseudosphaeriales and the family Pseudosphaeriaceae are in need of revision since the genus *Pseudosphaeria* upon which they are based was transferred to the Dothioraceae by Thiessen and Sydow (1917) and the family placed in the Myriangiales. He suggested at this time that a new order, the Pleosporales, be erected for those genera having the *Pleospora*-type of centrum development.

Luttrell (1955) established a new subclass of Ascomycetes known as the Loculoascomycetes and described under this subclass a new order, the Pleosporales containing the family Pleosporaceae. He recognized the family Pseudosphaeriaceae, but placed it in the Dothideales.

Ainsworth and Bisby (1954) list *Apiosporina collinsii* in the Meliolaceae under the Erysiphales and in the Hyalodidymae of the Sphaeriales.

Clements and Shear (3rd ed. 1957) list the species three times; once under the family Perisporiaceae and twice in the Sphaeriaceae, in the Hyalodidymae and the Phaeodidymae.



Müller and von Arx (1962), not recognizing the classification of Luttrell, classify *Apiosporina collinsii* in the family Venturiaceae within the order Pseudosphaeriales.

C. *Apiosporina morbosa* (Schw.) v. Arx

1. Developmental Studies

Farlow (1876) described the presence of mycelial threads of *Apiosporina morbosa* in fan-shaped masses, spreading from the cambium of the *Prunus* stem toward the cuticle. He reported that, "as spring advances, the mycelial threads increase in size, burst through the bark, and then form the dense pseudo-parenchymatous tissue characteristic of the Pyrenomycetes when about to fructify". The appearance of the exposed knot was described as being dark brownish-green.

This author gave the following account of the development of spore producing structures. Small, hemispherical protuberances occur on the surface of the knot which are the beginnings of the perithecia. The whole surface of these protuberances becomes covered with frequently simple, but occasionally branched, filaments. Spores are borne at the tip or to one side of the tip of the terminal "joint" of the filament, and in some cases are produced on some of the lower "joints". The spores are 0.006 mm in length, ovate and pointed at the lower end. These spores or conidia are similar to those found





in the genus *Cladosporium*. Farlow stated that, "the conidia which we have just described spring directly from the surface cells of the perithecia. They continue to bear their spores until the latter part of summer, when they begin to dry up, and, as winter sets in, one finds only their shrivelled remains".

Spore production in the asci of the fungus occurs during the winter. It was found that the perithecia contained only hyaline asci in early winter and by the middle of January the spores began to ripen. In February they were found in "perfection". There are eight spores in each ascus, often arranged in a regular row. The asci are about 0.12 mm long and abruptly contracted at the base. They release their spores through a pore at the tip. Paraphyses, longer than the asci, unbranched and club-shaped, are associated with the asci.

The ascospores measured 0.16 mm to 0.02 mm in length and 0.008 mm to 0.010 mm in breadth and are two-parted; one division uniformly smaller than the other and only a quarter to a third as long. The smaller end is invariably pointed downward. The ascospores are transparent and "slightly granular". Farlow said that, "the first germinal tube grows invariably from the larger of the two divisions of the spore, and generally at the end farthest removed from the smaller division; a second tube grows from the smaller end; and not unfrequently others grow from the sides of the larger division".





The production of stylospores, spermagonia and pycnidia by the fungus was also reported. The stylospores are of the form found in the genus *Hendersonia*, but they do not seem to belong to any of the described species. The spermagonia are like the perithecia, but contain slender filaments with incurved tips which easily break off and fill the central cavity. The pycnidia are triangular in section and lined with delicate filaments which end in oval, hyaline bodies. These oval bodies are produced in great quantity in a jelly-like substance which oozes out of the cavities to release the spores. Farlow did not suggest a function for the spores produced by any of these structures.

## 2. Studies on the Host-Pathogen Relationship

The most thorough studies of the effects of *Apiosporina morbosa* on its host, *Prunus* species, have been conducted by Stewart (1914) and Koch (1935).

Stewart describes the knots as arising from primary infections (stem infected directly by spores) or from secondary infections (spreading of the fungus in the stem from pre-existing knots). He states that the greatest disturbance occurs in the vicinity of the leaf gaps because of the large amount of parenchyma present in such areas.

Stewart reports a great deal of hypertrophy and hyperplasia occurring in the stem due to the presence of



the fungus. The greatest increase in cell number and size occurs in the multiseriate rays of the stem. The vascular cambium maintains its position between the xylem and the phloem except in the regions opposite the rays where it is pushed out and isolated in the cortex. Where this occurs, isolated xylem elements in the form of scalariform tracheids are produced in the cortical portions of the knot.

Koch substantiates the occurrence of hypertrophy and hyperplasia as reported by Stewart, particularly in the regions of the medullary rays. He states that, "normal medullary rays in twigs of *Prunus domestica* are usually multiseriate and as soon as invasion of these rays occurs in the cambial region, instead of forming the usual proportion of xylem elements and ray tracheids the cambium produces a relatively large number of parenchyma cells which are also many times their usual size". Koch believes this production of parenchyma to be responsible for the displacement of the cambium outwardly into the cortical tissues.

Koch reports that as infections age, the trachea become filled with gum and that gum pockets develop from the dissolution of parenchyma in affected medullary rays. This gum production is accompanied by the degeneration of adjacent fungus mycelium. The tissues of the host and the pathogen mycelium within the knot die during the winter or dormant period, while at the boundaries of the knot the mycelium spreads into previously uninfected



tissues.

### 3. Review of the Classification of *Apiosporina morbosa*

*Apiosporina morbosa* (Schw.) v. Arx has been described under a number of different names. It was first described as *Sphaeria morbosa*, a member of the Sphaeriaceae, by Schweinitz in 1821. Subsequently a number of names were suggested, but none was considered generally acceptable until Saccardo (1891) assigned the organism to the Dothideales under the name *Plowrightia morbosa* (Schw.) Sacc.

Thiessen and Sydow (1918) placed this fungus in their new order the Pseudosphaeriales and renamed it *Dibotryon morbosa* (Schw.) Theiss. & Syd.

Other names given to fungus are *Cucurbitaria morbosa* (Schw.) Ellis in 1881, *Othiella morbosa* (Schw.) Sacc. by Cooke in 1882 and *Botryosphaeria morbosa* (Schw.) Sorauer in 1921.

Koch (1935) realized that the fungus was not typically sphaeriaceous, but pseudosphaeriaceous in character and suggested that it was most advisable to use the name *Dibotryon morbosa* although he found it questionable whether Theissen and Sydow were justified in considering the Pseudosphaeriales as a separate order.

Miller (1938) believed this species to belong in the Pseudosphaeriales and placed it there in 1949.

Martin (1950) gives this species as a representative member of the Dothideaceae.







Ainsworth and Bisby (1954) list *Dibotryon morbosa* with other genera of the Dothideaceae and Phyllachoraceae under the Dothideales and suggest that it may belong in the Sphaeriales.

In 1954, von Arx examined this fungus more closely and named *Apiosporina morbosa* (Schw.) v. Arx. Müller and von Arx (1962) place this species in the Venturiaceae of the Pseudosphaeriales.

D. *Apiosporina harunganae* Hansf.

*Apiosporina harunganae* was described by Hansford (1947) as a new species and placed in the Sphaeriaceae. The fungus was discovered on the leaves of *Harungana madagascariensis* Poir. in Kisubi, Entebbe, Uganda.

Hansford described the pale brown mycelium as entirely superficial on the leaves and as having hyphopodia. The perithecia of this fungus are loose, irregularly dispersed, blackened, globose or somewhat papillate structures. The perithecial wall consists of a single layer of parenchymatous cells forming an outer wall and an inner wall of indistinct, hyaline cells. There are many, pointed setae scattered irregularly over the surface.

The asci are ellipsoid in shape, with a short stalk and are aparaphysate. The ascospores are two or three-seriate, clavate, smooth, medium septate, slightly



constricted in the centre with a wide apical cell. They are hyaline at first, then pale olive. There is no conidial stage mentioned.



## MATERIALS AND METHODS

### A. Materials

Sprague and Heald (1927) showed *Apiosporina collinsii* (Schw.) von Höhnelt to be a perennial parasite in its host, *Amelanchier* spp. It was necessary, therefore, to collect and examine stems, buds, leaves, flowers and fruits of the host in order to arrive at a thorough understanding of the development of the fungus.

Approximately eighty collections were made locally from June 1964 until December 1965. Infected and non-infected host material was collected and assigned a collection number when permanent slides were to be made.

Herbarium collections were also examined. This material included 73 Canadian collections and 65 collections from the United States.

The species of *Amelanchier* common to Alberta and upon which the study was conducted was *A. alnifolia* Nutt. Collections of other infected host species were obtained from the Mycological Herbarium, Department of Agriculture, Ottawa, Ontario, Canada; Cryptogamic Herbarium, The New York Botanical Garden, New York, New York, U.S.A. and the Mycological Herbarium, Department of Botany, The University of British Columbia, Vancouver, British Columbia, Canada. Other species of the host examined were; *A. arborea* (Michx. f.) Fern., *A. canadensis* (L.) Medic., *A. florida* Lindl.,





*A. intermedia* Spach, *A. oreophila* A. Nelson (= *A. utahensis* Koehne) and *Amelanchier* spp.

Material of *Apiosporina morbosa* (Schw.) v. Arx. was obtained from the Mycological Herbarium, Department of Agriculture, Ottawa, Canada for examination. A list of the hosts infected by this species is to be found in Appendix C (page 134).

## B. Methods of Study

### 1. Maceration and Staining Techniques

To study the internal fungus mycelium in leaves, petioles, buds and stems of the host, the material was boiled in 10% KOH for 10 minutes and then washed in running water to remove excess KOH. Small portions of the material were then placed in a drop of cotton blue plus lactophenol on a microscope slide and spread by applying pressure on the cover glass. The fungus mycelium stained darker than the host tissues and could be followed among the host cells.

While maceration of host material with KOH proved satisfactory for the examination of internal mycelium, this method gave poor results when the contents of the ascocarps were to be examined. It was necessary to use a stronger macerating agent to soften the walls of the ascocarps and allow even spreading of their contents. For this purpose,



leaves bearing ascocarps were placed in Jeffrey's solution (Johansen 1940) for 10 minutes. The material was then washed in running water to remove excess acid. Individual ascocarps could be removed from the leaf by means of a needle and a dissecting microscope, placed on a microscope slide, stained and squashed.

It was found that cotton blue in lactophenol did not serve as a satisfactory stain for this material. Other stains tried were phloxine B (saturated solution in water), aceto-carmin (Sass 1940), aceto-orcein (George T. Gurr Ltd.), iodine, ammoniacal Congo red with 10% ethanol substituted for water (Richardson and Morgan-Jones 1964) and blue writing ink. Best results were obtained using the Congo red or the ink methods. The latter was prepared by mixing 2 parts ink (Parker Super Quink, Permanent Blue-Black) with 2 parts glycerine and 1 part lactophenol. As the asci and pseudoparaphyses tended to stain very deeply if left in this solution, it was necessary to remove the stain after 5 - 10 minutes and replace it with glycerine or lactophenol.

## 2. Free-hand Sections

Leaves bearing mature ascocarps were collected in June. Portions of these leaves were placed in melted Polyethylene glycol 20,000 (Fisher Scientific Company) on a microscope slide in preparation for free-hand sectioning by a method described by Taylor (1957). When the wax had hardened, sections were made by cutting with a razor blade



under a dissecting microscope. In this manner, sections the thickness of a whole ascocarp could be obtained. These sections were floated in water until the wax dissolved. The material could then be mounted in water on a microscope slide for observation of the release of the ascospores.

### 3. Moist Chamber Methods

To ascertain that the ascospore release observed was natural and not caused by artificial factors due to the pressure of a coverslip and an aqueous medium, portions of leaves with mature ascocarps were placed in moist chambers and the spores collected. This was achieved by placing the material on a piece of moistened pith in the bottom of a Van Tieghem cell. Released spores were collected in a hanging drop of distilled water suspended above the material on a coverslip or on a coverslip which was lightly smeared with Vaseline. To successfully collect the spores, the water drop or the greased coverslip had to be placed at a height no greater than 5 millimeters above the material. Otherwise the released spores could only be found on the base of the cell.

### 4. Spore Germination Methods

Conidiospores and ascospores germinated readily in hanging drops of distilled water in Van Tieghem cells. Germinating ascospores were occasionally found on the lightly greased coverslips. Germinating conidiospores







were frequently found in the subiculum on the undersurface of the leaves when examined during the summer.

Ascospores germinated within 24 hours; conidiospores within 48 hours.

## 5. Spore Collection Method

A slide, lightly greased with Vaseline, was set in a vane spore collector mounted on a post five feet above ground. The collector was set up in the interior of a thicket of heavily infected shrubs at the University of Alberta Botanic Gardens, near Devon, Alberta. Fresh slides were placed in the collector every 3 days over a period from May 14, 1965 to July 15, 1965.

The slides were stained with cotton blue in lactophenol to examine for the presence of spores.

## 6. Preparation of Permanent Slides

### a) Sliding Microtome Methods

Stem material was cut into sections approximately 4 centimeters in length and fixed in a 70% ethanol solution of FAA (Johansen). Longitudinal and cross sections were cut with a sliding microtome (American Optical Company, Model 860) at thicknesses of 10 - 30 microns. These sections were stained with Safranin and Fast Green according to the following schedule.

1) Water

1 hour



- 2) 50% ethanol 10 minutes
- 3) Dip briefly in Safranin in 50% ethanol
- 4) Destain briefly in 50% ethanol
- 5) 70% ethanol 1 minute
- 6) 95% ethanol 1 minute
- 7) Dip in Fast Green in 95% ethanol
- 8) Destain briefly in 95% ethanol
- 9) Dip briefly in absolute ethanol
- 10) Absolute xylene 5-10 minutes

The sections were passed by means of forceps from solution to solution contained in petri dishes. When staining was completed, the sections were permanently mounted on microscope slides using Permount (Fisher Scientific Company) as a mounting medium. The results obtained were of little value as the fungus mycelium was closely packed between the host cells making it exceedingly difficult to distinguish it from the walls of the host cells.

Better results were obtained when the stem material was macerated and stained as previously described. It was found that with care, it was possible to strip away portions of the material and examine it for the presence of the fungus by squash techniques.

#### b) Embedding and Rotary Microtome Methods

Fresh material was fixed for 24 hours. At the



beginning of this study a number of different fixatives were used. These included; FAA (Johansen), FPA (Johansen), CRAF 2 and CRAF 3 (Sass), chrom-acetic solution (Johansen) and Newcomer (1953) fixatives.

The best results were obtained with FAA, FPA and chrom-acetic fixatives and these were employed throughout the remainder of the investigation. It was found that FAA and FPA penetrated the tissues rapidly, but that penetration was much slower with chrom-acetic solution. Material fixed by this method had to have the air removed by means of a vacuum pump in order to speed fixation. Newcomer's fixative was discarded as it caused extreme plasmolysis of the leaf cells. The fixatives containing chromic acid were used for preserving nuclear detail, however, the results were always poor and as the study was not concerned with the genetics of the fungus, the use of these fixatives was abandoned.

The embedding technique of Johansen (1940) was followed with certain modifications and found to give satisfactory results. From the fixatives (FAA, FPA) containing 70% ethanol the material was carried through the following series.

- |   |           |
|---|-----------|
| 1) 70% tertiary butyl alcohol solution  | overnight |
| 2) 85% tertiary butyl alcohol solution  | 2 hours   |
| 3) 95% tertiary butyl alcohol solution  | 2 hours   |
| 4) 100% tertiary butyl alcohol solution | 2 hours   |







- 5) Absolute tertiary butyl alcohol 2 hours
- 6) 50/50 absolute tertiary butyl alcohol -  
used Fisher Tissuemat (m.p. 56.5°C.)  
placed in warming oven overnight or until  
all traces of alcohol had disappeared
- 7) Used Tissuemat 4 hours
- 8) Pure Tissuemat 4 hours
- 9) Pure Tissuemat 4 hours

The hot Tissuemat containing the material was then quickly poured into paper boats (1.5 x 6 cm). The material was arranged by means of a hot needle and the Tissuemat was allowed to cool until a thin film appeared over the surface of the wax. The boat was then submerged in ice water to uniformly solidify the Tissuemat. The embedded material was mounted on wooden blocks and the excess Tissuemat trimmed with a razor blade in preparation for microtoming.

To study the internal mycelium in the buds, flowers, fruits, petioles and leaves of the host and the developmental changes in the external fungus parts, the material was sectioned with a rotary microtome (American Optical Company, Model 815). Transverse and longitudinal sections were cut at a thickness of 10 - 12 microns. This range was found to be most satisfactory after examination of sections ranging from 5 - 20 microns which were made in the initial stages of the investigation. The sections



were affixed to microscope slides with Haupt's adhesive.

Many problems were encountered in selecting suitable stains to be used with the sectioned material. Stains investigated were Crystal Violet, Delafield's Haematoxylin, Heidenhain's Iron Haematoxylin and Safranin and Fast Green. In the preparation of permanent slides the best results were obtained using Heidenhain's Iron Haematoxylin and the Safranin and Fast Green methods. The former showed the host cells, external fungus parts and the internal foliar mycelium quite well. The latter was best for differentiating the ascocarp walls from the contents of the centrum.

When Heidenhain's Iron Haematoxylin stain was used it was prepared as suggested by Johansen (1940) except that ethyl cellosolve (Fisher Scientific Company) was used in place of methyl cellosolve. In staining the material the following schedule was used.

- 1) Absolute xylene (until Tissuemat disappeared)
- 2) 50/50 absolute xylene-absolute ethanol 4-5 minutes
- 3) Absolute ethanol 4-5 minutes
- 4) 95% ethanol 4-5 minutes
- 5) 85% ethanol 4-5 minutes
- 6) 70% ethanol 4-5 minutes
- 7) 50% ethanol 4-5 minutes
- 8) 35% ethanol 4-5 minutes
- 9) Rinse in tap water, followed by distilled
- 10) 4% Ferric ammonium sulphate 1-2 hours



- |   |                       |
|---|-----------------------|
| 11) Rinse in tap water, followed by distilled |                       |
| 12) Heidenhain's haematoxylin                 | 2-24 hours            |
| 13) Rinse in tap water, followed by distilled |                       |
| 14) 2% Ferric ammonium sulphate               | $\frac{1}{4}$ -1 hour |
| 15) Rinse in tap water, followed by distilled |                       |
| 16) 35% ethanol                               | 4-5 minutes           |
| 17) 50% ethanol                               | 4-5 minutes           |
| 18) 70% ethanol                               | 4-5 minutes           |
| 19) 85% ethanol                               | 4-5 minutes           |
| 20) 95% ethanol                               | 4-5 minutes           |
| 21) Absolute ethanol                          | 4-5 minutes           |
| 22) 50/50 absolute xylene-absolute ethanol    | 4-5 minutes           |
| 23) Absolute xylene                           | 4-5 minutes           |

When staining with Safranin and Fast Green, the following procedure was employed.

- |   |             |
|---|-------------|
| 1) Absolute xylene (until Tissuemat disappeared)  |             |
| 2) 50/50 absolute xylene-absolute ethanol         | 4-5 minutes |
| 3) 95% ethanol                                    | 4-5 minutes |
| 4) 85% ethanol                                    | 4-5 minutes |
| 5) 70% ethanol                                    | 4-5 minutes |
| 6) Safranin in 70% ethanol (until deeply stained) |             |
| 7) 85% ethanol                                    | 1-2 minutes |
| 8) 95% ethanol                                    | 1-2 minutes |
| 9) Fast Green in 95% ethanol (quick dip)          |             |
| 10) 95% ethanol (dip)                             |             |







- 11) Absolute ethanol (dip)
- 12) 50/50 absolute xylene-absolute ethanol 1-2 minutes
- 13) Absolute xylene

The procedures described by Johansen (1940) were followed when staining with Crystal Violet or Delafield's Haematoxylin. On completion of staining, the material was mounted in Permount (Fisher Scientific Company).

Very little could be seen in cut sections of buds. Better results were obtained when this material was macerated by the previously described method using KOH. The embryonic leaves could be dissected out and the presence of the mycelium was shown by staining with cotton blue in lactophenol.

## 7. Photography

Photomicrographs were taken with a Zeiss Photomicroscope (55575) using Adox KB 14 film or a Leitz Wetzlar Photomicroscope (595655) using Kodak Contrast Process Ortho Film (Canadian Kodak Company). The films were developed in a 1:1 Dektol (Eastman Kodak Company) -tap water solution or in a 1:20 Perinal (PERUTZ) -tap water solution at 68°F. They were rinsed in cold running water for 30 minutes, immersed in Photo-flo (Eastman Kodak Company) for 30 seconds then dried.

Prints were made on Kodak Kodabromide single weight F-4 paper (Canadian Kodak Company) and developed in



the same manner as the films. All photomicrographs were enlarged with a Beseler Enlarger (Model 45MX).

## 8. Glossary

Discussion of the life history and classification of this fungus involves the use of a number of controversial terms. The following glossary lists these terms with the definitions applied to them throughout this investigation.

apothecium	- an ascocarp with an exposed hymenium; varying in shape from sponge to cup or disc-like forms.
ascocarp	- a definite structure containing asci.
ascostroma	- a stromatic ascocarp with asci arising directly in locules within the stroma; varying from pulvinate to perithecioid forms.
bitunicate ascus	- an ascus having two distinct walls; a thick outer wall and a thin, elastic inner wall.
centrum	- the total contents of an ascocarp.
cleistothecium	- a closed, spherical ascocarp containing globose or broadly club-shaped asci produced at various levels within the ascocarp; when mature the ascocarp and asci



walls disintegrate to release the mature spores.

- Loculoascomycetes - a subclass of the Ascomycetes in which bitunicate asci are produced within ascostromata.
- ostiole - a neck-like structure of an ascocarp, lined with paraphyses and opening by means of a pore.
- perithecium - a spherical or flask-shaped ascocarp consisting of a wall which arises as the result of a sexual stimulus and containing a centrum in which unitunicate asci are formed; opening by a pore, an ostiole or by disintegration.
- pseudoparaphyses - a fan-shaped group of hyphae which appear at the apex of the inner wall and grow downward to become attached to the cells lining the base.
- pseudothecium - a uniloculate ascostroma, perithecioid in shape.
- Pyrenomycetes - a group of Ascomycetes which produce unitunicate asci within perithecia; does not have taxonomic rank.
- stroma - a compact somatic structure, pseudoparenchymatous in nature, on or in which ascocarps are formed.





- subiculum - a loose hyphal mat on or in which  
ascocarps arise.
- unitunicate ascus - an ascus having only one wall.



## OBSERVATIONS

A. The Biology of *Apiosporina collinsii* (Schw.) von Höhnelt

## 1. The Ontogeny of the Fungus

## a) The Internal Mycelium

Conidiospores and ascospores of *Apiosporina collinsii*, when mature, germinate readily under moist conditions within 48 hours. The pathogen gains entrance to its host through fissures in the bark or at leaf axils. The spores produce a single germ tube from the apical cell of the ascospore (Figure 27, page 64) or from any point of the conidiospores (Figure 1, page 53). On rare occasions, two germ tubes may be produced by the conidiospores (Figure 2, page 53). The spores swell slightly and the germ tube can be seen extending through a rupture in the spore wall.

The germ tube branches shortly after its appearance (Figure 1, page 53) and continues to elongate and produce cross walls forming cells. The mycelium formed is composed of small, rectangular, rarely branching, hyaline cells. The cells vary in length, but are on the average 6 microns wide. The contents of the cells appear granular and may contain a number of oil droplets. This mycelium can be located in the stem mainly in the cortex and phelloderm regions, occasionally in the wood. It grows toward the apex of the infected stem and from this source grows out into the buds, petioles, leaves and flowers of the host.



In the petioles the mycelium can be traced along the tracheids into the leaves where it travels along the vascular tissues of the veins, then spreads throughout the mesophyll cells of the laminae. Branches of the hyphae in the petioles can be seen surrounding the non-vascular cells and growing between the xylem elements. In the leaf lamina, the mycelium is mainly associated with the spongy mesophyll and spreads only slightly into the palisade tissue.

Throughout the spongy mesophyll region of the leaves, the mycelial cells vary greatly in size, measuring  $8.0 - 16.0 \times 1.6 - 3.2 \mu$ . They may be rectangular to irregular in shape depending on the amount of branching (Figures 7 and 8, page 55). The internal mycelium in the leaf, when fully developed, forms a highly branched network surrounding the host cells.

#### b) Conidial Development

Conidiophores are the first external structures to appear on the undersurface of the infected leaves. They appear soon after the leaves unfold in the spring and may cover the entire undersurface of the leaf by the end of June.

The mycelium emerges between the lower epidermal cells or through the stomata, then spreads horizontally to form a single layer of multiseptate hyphae on the leaf surface. This layer is composed of cells measuring  $3.2 - 4.8 \times 6.4 - 11.2 \mu$  which are first hyaline, then olive

Figure 1. (X500) Germinating conidiospore of *Apiosporina collinsii* showing branching of the germ tube.

Figure 2. (X500) Conidiospores of *Apiosporina collinsii* showing the rare production of two germ tubes by a germinating spore.

Figure 3. (X500) Apical cell of a conidiophore acting as a spore.

Figures 4 - 6. Conidiophores of *Apiosporina collinsii* from KOH macerated material.

Figure 4. (X250) Conidiophores on lower epidermal cells of the host. Arrow indicates a bulbous basal cell.

Figure 5. (X350) Conidiophore with prominent oil globules in the cells.

Figure 6. (X450) Cluster of conidiophores showing the superficial hyphal layer from which they arise (arrow).







-brown in color. This superficial layer gives rise to the single or branched conidiophores. The conidiophores consist of a bulbous basal cell and three to five, smooth-walled, cylindrical cells measuring  $3.2 - 4.8 \times 11.2 - 24.0 \mu$  and are olive-brown in color (Figures 4 - 6, page 53). The cells of the conidiophores contain prominent oil globules (Figure 5, page 53).

Conidiospores are produced by any of the conidiophore cells, with the apical cells being most active in spore production. The spores when first produced are hyaline and thin-walled, but soon become thick-walled and olive-brown in color. The majority of the spores are  $7 \times 14 \mu$  in size, but they vary from  $6.2 - 9.3 \times 7.8 - 21.7 \mu$ . The spores are typically lemon-shaped (Figures 1 and 2, page 53) but can be ovoid to cylindrical or irregular in shape. The terminal cell of the conidiophore often separates from the adjoining cells by the production of a double wall and it may also act as a spore (Figure 3, page 53).

These structures can be easily examined in KOH macerated material. It is unnecessary to stain this material because it is dark olive-brown in color.

#### c) Subiculum Development

Following the appearance of the conidiophores, the subicular hyphae begin to develop. These hyphae are produced by the same superficial layer of multiseptate

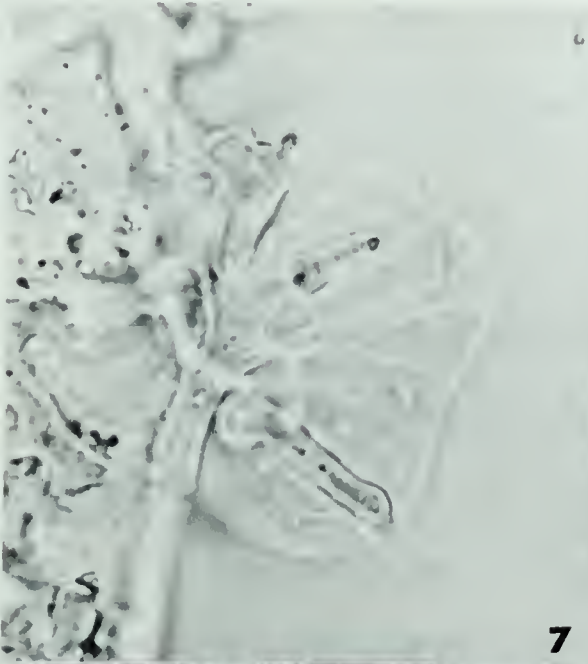
Figures 7 - 10. Internal mycelium of *Apiosporina collinsii*.

Figures 7 and 8. (X1600) KOH macerated material showing the hyaline mycelium located around the spongy mesophyll cells of the leaf.

Figure 9. (X1000) Cross section of an infected leaf showing the presence of the hyaline mycelium (arrow) between the fibre cells (F) of the leaf midrib.

Figure 10. (X900) Cross section of infected stem showing the mycelium (arrow) between the fibre cells (F) in the stem cortex.

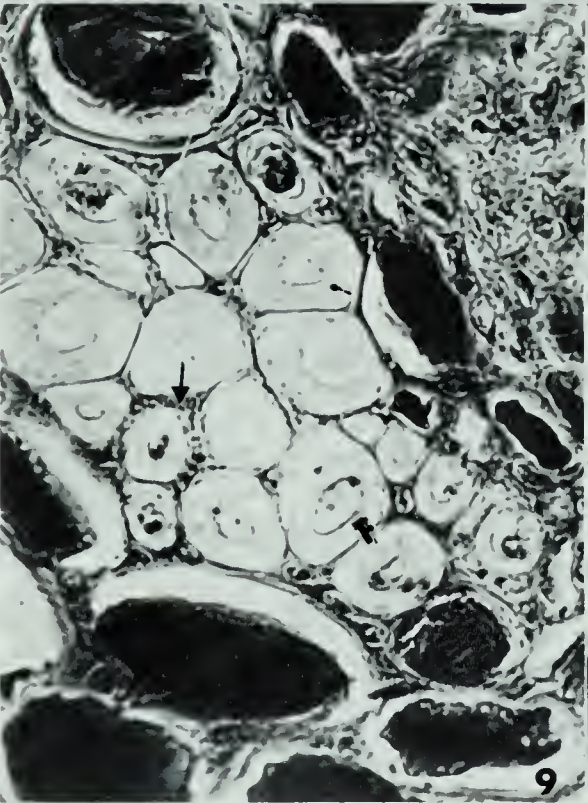




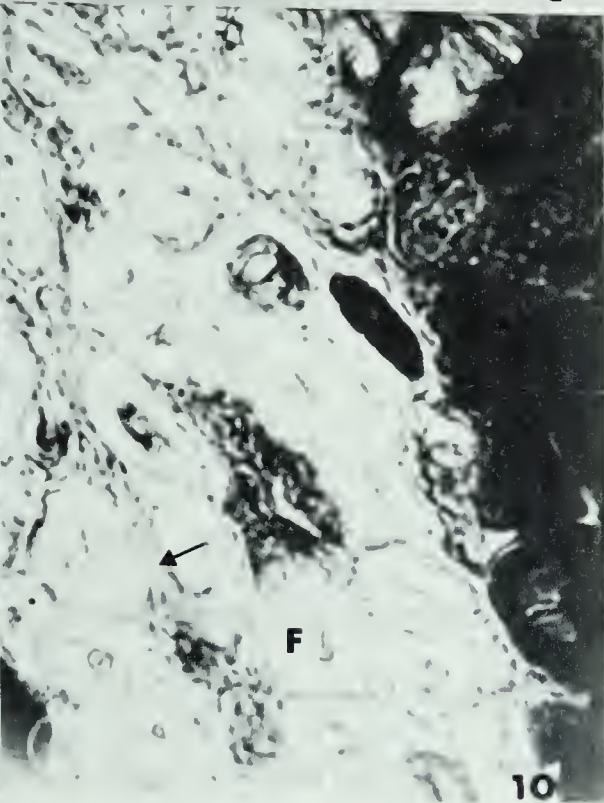
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9



10

F





hyphae that gives rise to the conidiophores. This layer produces bulbous cells measuring 8.0 - 9.6  $\mu$  in diameter (Figures 11 and 13, page 57) which branch to form the subicular hyphae. The subiculum is composed of frequently branched, olive-brown hyphae not unlike the conidiophores in appearance.

#### d) Pseudothecial Development

The pseudothecia begin developing in the subiculum by the end of June or the first part of July. When mature, the pseudothecia are black, globose, crowded bodies occasionally laterally fused, 150 - 250  $\mu$  in diameter (Figures 17 and 18, page 58). They first appear as small knots of smaller-celled, more closely associated subicular hyphae (Figures 11 - 13, page 57). By repeated branching and cell division the subicular cells give rise to the walls of the pseudothecia.

The pseudothecial wall is composed of two zones (Figure 24, page 62); an outer zone consisting of three or four layers of brown-black, pseudoparenchymatous, thick-walled cells; and an inner zone of thin-walled, hyaline cells three or four rows deep. The inner region can be distinguished by staining sectioned material with Safranin-Fast Green or Heidenhain's Haematoxylin.

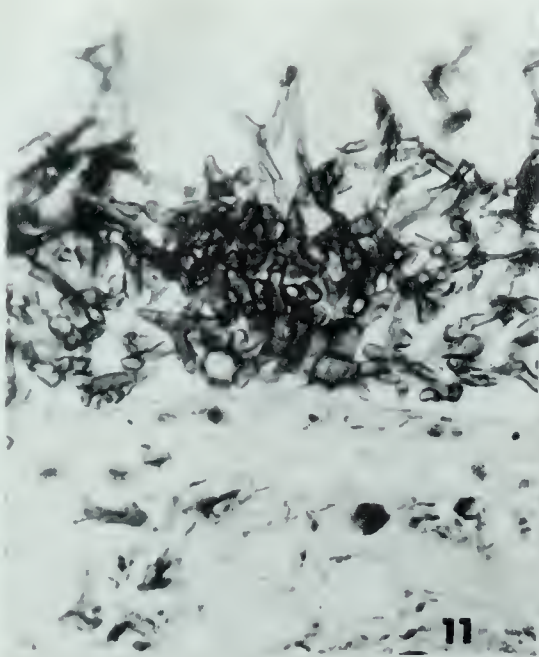
Occasionally simple hyphal appendages can be found on the pseudothecia (Figure 24, page 62). Their occurrence is rare as they appear only when occasional

Figures 11 - 13. Cross sections of infected leaves showing the knots formed in the subiculum which give rise to the pseudothecia of *Apiosporina collinsii*.

Figure 11. (X500) Aggregation of subicular hyphae to form pseudothecial knot.

Figures 12 and 13. (X500) Pseudothecial knots enlarging and taking on globose shape.

Figure 14. (X625) Cross section of an immature pseudothecium showing the pseudoparenchymatous stage in centrum development.



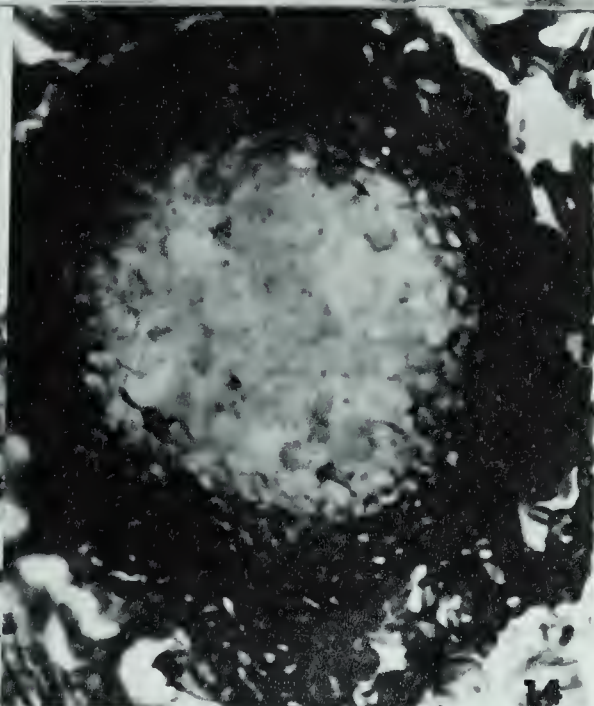
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Figures 15 and 16. Cross sections of pseudothecia of *Apiosporina collinsii* stained with Heidenhain's Haematoxylin showing pseudoparaphysate centra.

Figure 15. (X350) Pseudoparaphyses arising at the apex of the centrum and growing toward the base. The tips of the pseudoparaphyses stain deeply at this stage.

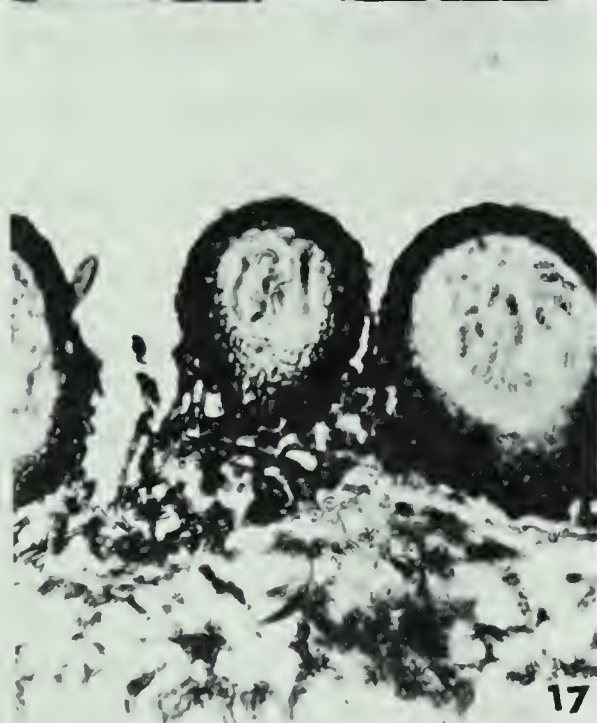
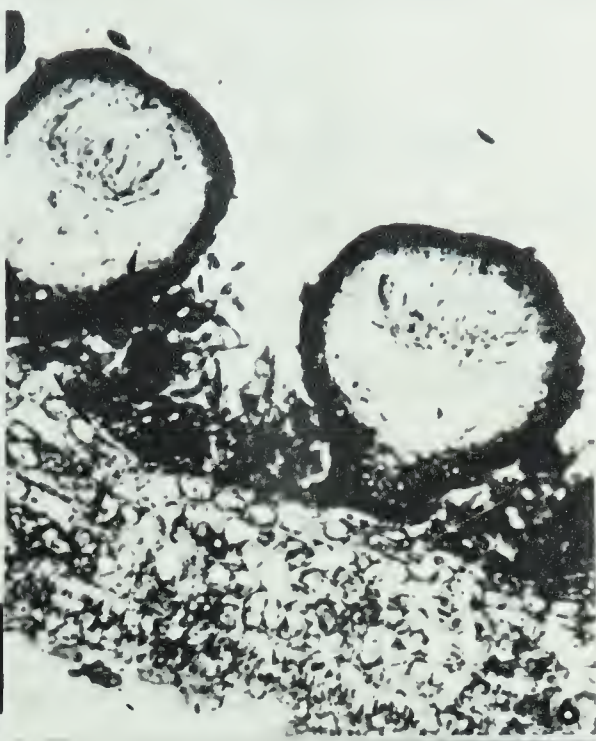
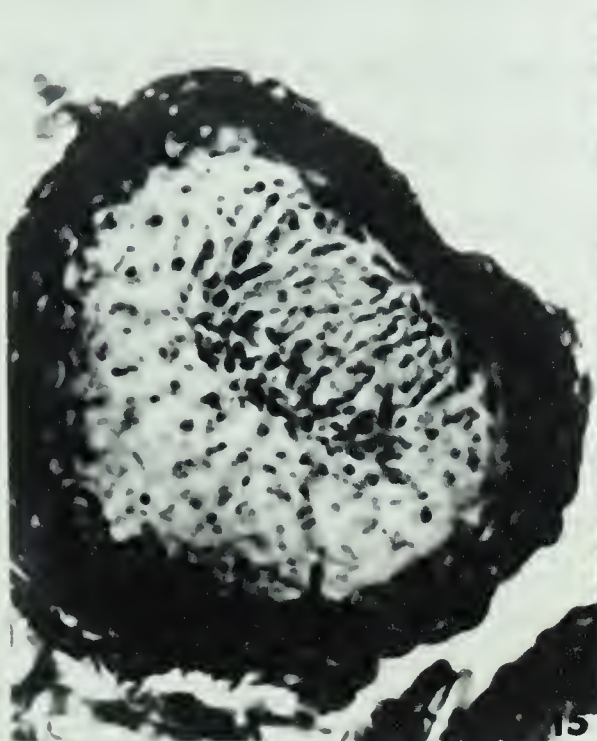
Figure 16. (X150) Pseudoparaphysate centra in immature pseudothecia.

Figures 17 and 18. Cross sections of mature pseudothecia of *Apiosporina collinsii* stained with Safranin and Fast Green.

Figure 17. (X125) Mature pseudothecia with centra composed of asci and pseudoparaphyses.

Figure 18. (X150) Laterally fused pseudothecia.









subicular branches remain free from the hyphal knots which form the pseudothecial wall.

The centrum, or the contents of the ascocarp, goes through a number of developmental stages before the production and release of mature ascospores.

In its earliest developmental stage, the centrum consists of thin-walled, hyaline, pseudoparenchymatous cells (Figure 14, page 57). Following this stage, a fan-shaped group of hyphae, the pseudoparaphyses (Figures 15 and 16, page 58), is produced at the apex of the centrum by the inner layer of wall cells. These actively growing, single or branched hyphae grow toward the base of the centrum where they fuse with the cells lining the base or with each other. The tips of the pseudoparaphyses stain deeply with Heidenhain's Haematoxylin distinguishing them from other parts of the centrum at this stage.

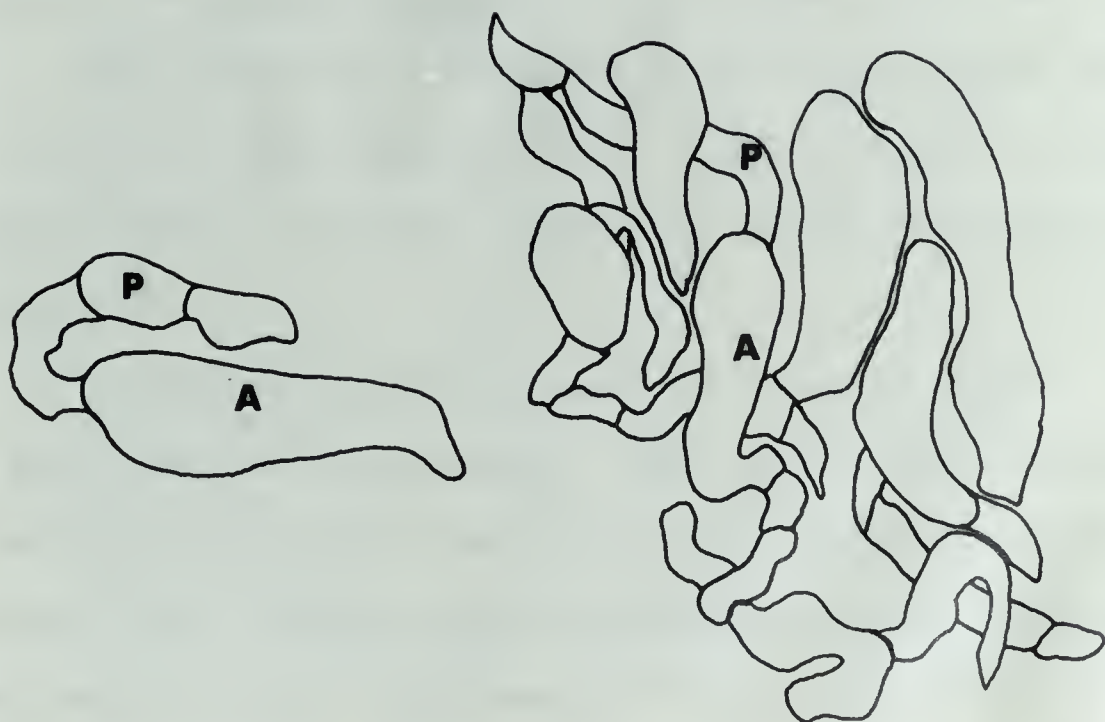
The asci begin development after the pseudoparaphyses have reached the base of the centrum. They develop from binucleate cells formed in the basal region of the centrum by fusion between the tips of the pseudoparaphyses and cells in this area. Squashes of ascocarp material at this stage of development frequently show developing asci with attached pseudoparaphyses (Figures 19 and 20, page 60). The asci grow upward among the pseudoparaphyses (Figures 22 and 23, page 62). When mature, the asci are short-stalked, bitunicate, cylindrical structures measuring  $6.4 - 8.0 \times 56.0 - 72.0 \mu$  con-

Figure 19. (X1000) Developing ascogenous cells with pseudoparaphyses attached. Stained with writing ink.

Figure 20. (X4000) Drawing of material in Figure 19. (P) pseudoparaphyses (A) ascogenous cells



19



20



taining eight, two-celled ascospores (Figure 21, page 62; Figure 29, page 65). The bitunicate nature of the ascus wall is difficult to observe. When stained with writing ink the two wall layers are faintly evident. The tips of the asci appear slightly thicker-walled and a dimple (Figure 29, page 65) can frequently be seen in the inner wall at the tip. These ascus tips do not stain with iodine; a test used to separate unitunicate and bitunicate asci. The bitunicate ascus wall is best seen in macerated material stained with ammoniacal Congo red. The outer wall frequently ruptures and spreads apart, showing the presence of the inner, thin wall which keeps the contents in place. Further evidence of an outer wall can also be seen when examining ascospore discharge. After the outer wall ruptures, a thin line can sometimes be noticed moving down the outside of the extended inner wall. This line likely represents the tip of the outer wall as it contracts toward the base.

The ascospores consist of a large apical cell and a much smaller basal cell. They are always arranged in a vertical line with the large cell toward the tip of the ascus. The spores begin development in the fall and mature over winter. They are at first elliptical in shape (Figure 21, page 62), and become ovoid when mature (Figures 25 - 29, pages 64 and 65). Mature ascospores have been observed germinating on glass slides from the spore trap by the end of May. The ascospores are hyaline

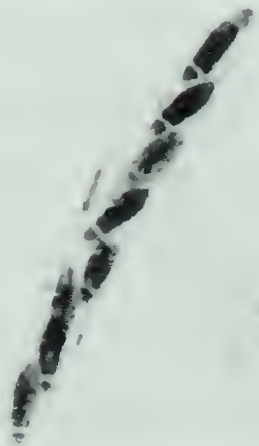
Figure 21. (X1000) Immature ascus containing elliptical ascospores. Stained with writing ink.

Figure 22. (X420) Portion of a squashed centrum showing immature asci among pseudoparaphyses. Stained with writing ink.

Figure 23. (X830) Mature pseudothecium showing bases of the pseudoparaphyses at the apex of the centrum. Stained with Heidenhain's Haematoxylin. (P) pseudoparaphyses

Figure 24. (X920) Wall of a mature pseudothecium showing the outer zone of olive - brown, isodiametric cells and the inner zone of flattened, hyaline cells. Arrow indicates an appendage on the pseudothecial wall which is formed by a branch of the subicular hyphae not incorporated into the wall. Stained with Saffranin and Fast Green.

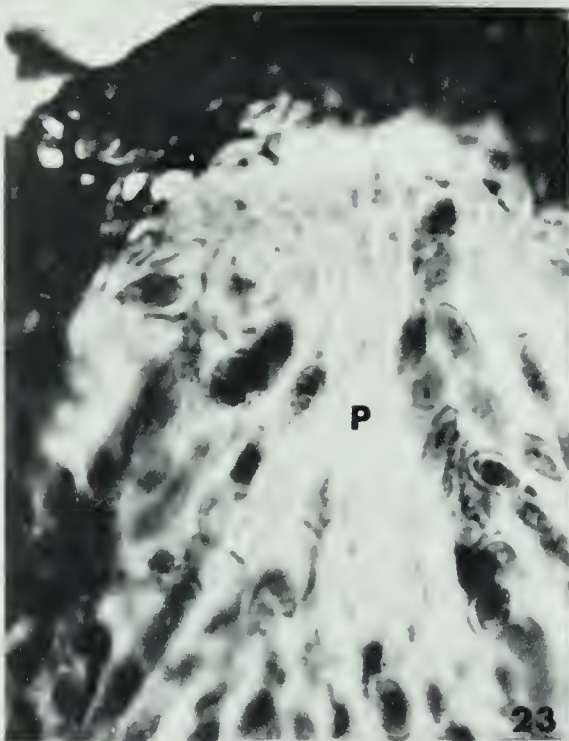




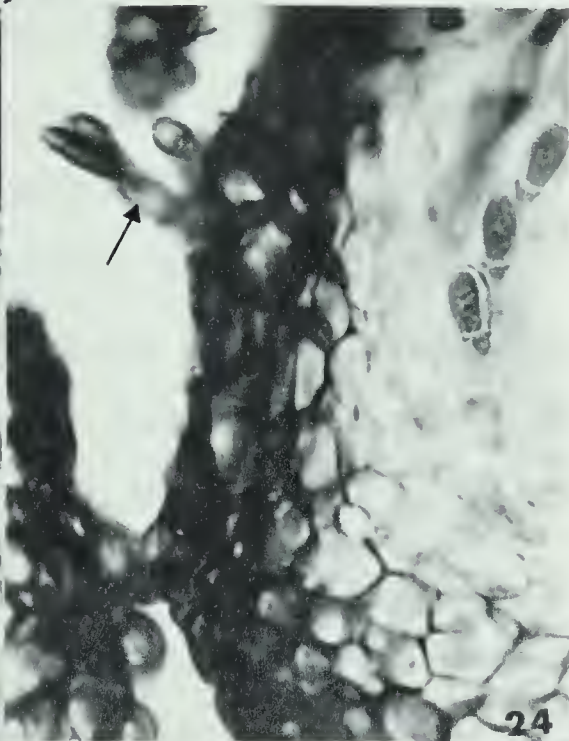
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24



when observed under white light and  $4 - 6 \times 12 - 15 \mu$  in size when mature.

e) Ascospore Release

Prior to the opening of the ascocarp, the pseudoparaphyses gelatinize, take-up moisture and swell to exert the pressure necessary for the splitting of the pseudothecial wall. The opening formed by a stellate splitting of the wall in the apical region is uneven and the torn edges tend to curl back. Once the opening is formed, the gelatinized pseudoparaphyses are exuded from the newly opened ascocarp as a drop of mucous-like substance (Figure 28, page 64) lacking hyphal organization.

When observed in the lab in an aqueous preparation, the asci are seen to move up and protrude through the break in the pseudothecial wall (Figure 28, page 64). As this can be seen only by examining whole ascocarps, it is impossible to tell if the moving up is due to extension of the asci alone, or whether other factors are involved. When fully extended, the outer ascus wall ruptures and the inner, elastic wall expands to extend through the ruptured outer wall. During the extension of the ascus, the ascospores gradually move toward the apex of the ascus. Once sufficient pressure is built up within the ascus, the uppermost spore pushes through the ascus tip and the spores are forcefully ejected one after the other. There is some evidence for the presence of an elastic pore in the tip of

Figure 25. (X165) Group of ascospores discharged into water drop in a Van Tieghem cell.

Figure 26. (X650) Mature, two-celled, hyaline ascospores of *Apiosporina collinsii*.

Figure 27. (X830) Drawing of germinating ascospores showing the rupturing of the apical cell wall by the germ tubes.

Figure 28. (X590) Aqueous mount of a free-hand section bearing a mature pseudothecium showing the extension of an ascus prior to spore discharge. Arrow indicates the gelatinized pseudoparaphyses released after splitting of the pseudothecial wall.



25



26



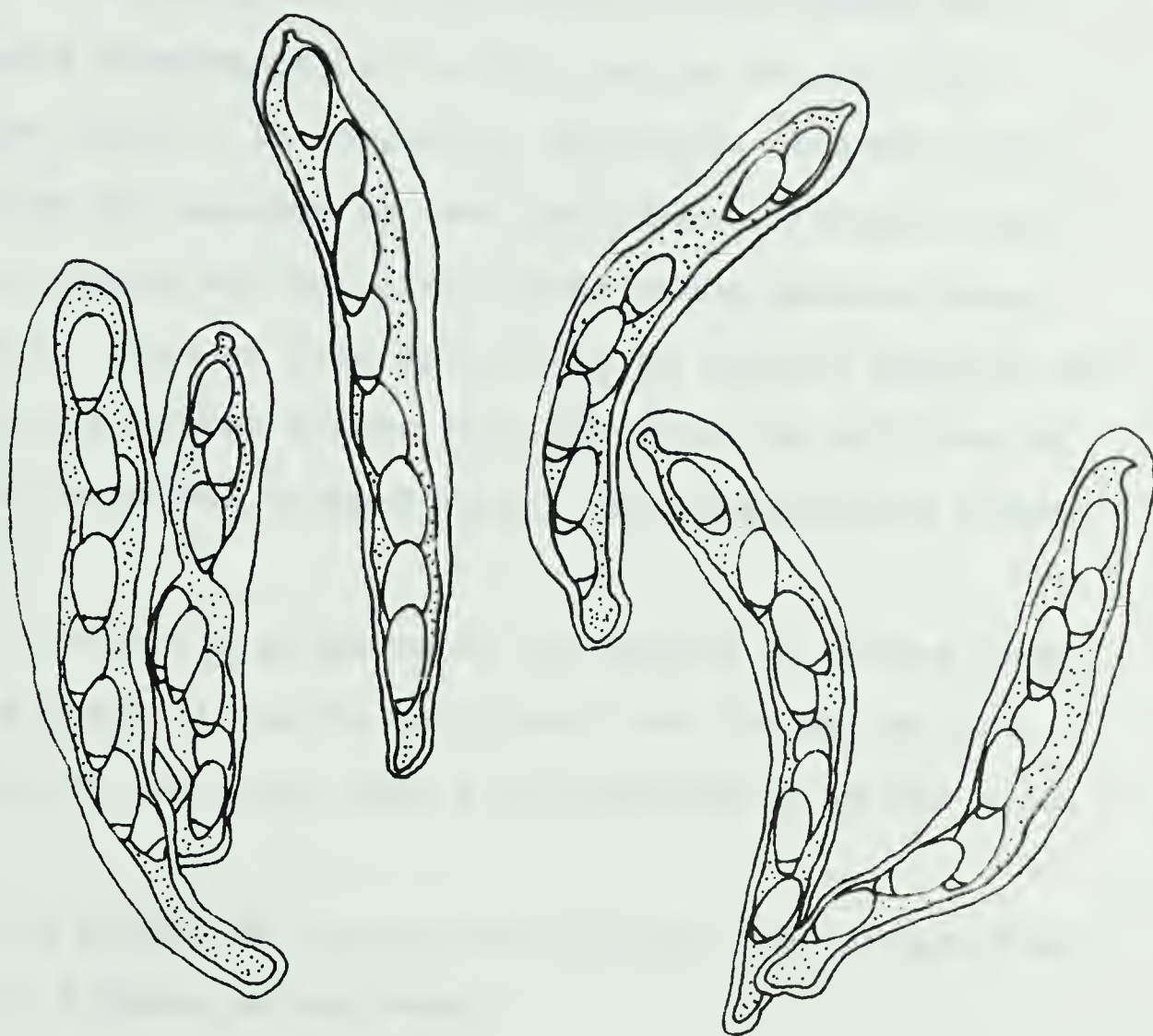
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Figure 29. (X1000) Drawing of mature asci showing the bitunicate nature of the walls and the "dimples" in the outer wall at the apices of the asci. Asci are slightly plasmolized due to the use of a strong macerating agent.







the inner ascus wall. On a number of occasions, spores appeared to be held in place with the apical, larger cell free of the ascus and the smaller, basal cell still within the ascus. Often, after the release of the spores the empty ascus appears to have a thin collar at the tip. Once spore release is completed, the ascus contracts and leaves room for another to take its place. Forceful discharge of spores did not always occur under aqueous mount conditions. Four or five spores may be rapidly ejected and the remaining spores either flow out with the epiplasm of the ascus or become trapped within the constricting ascus wall.

From the presence of ascospores on slides from the spore trap, it can be said that once the spores are released from the asci, they are disseminated by the wind.

## 2. The Effect of *Apiosporina collinsii* on *Amelanchier*

### a) Effects on the Stem

The main symptom of infection of *Amelanchier* species by *Apiosporina collinsii* is the formation of witches' brooms by the infected branches. These brooms are of two types: (i) a compact, thickly-branched, curved broom (Figure 30, page 67) and (ii) a looser, open type (Figure 31, page 67). Findings in the field indicate that the type of broom formed is dependent on environmental conditions.

The loose, open type of witches' broom is

Figure 30. Compact type of witches' broom commonly found in moist, shaded areas. Uninfected branches are present in the background.

Figure 31. Loose, open type of witches' broom commonly found in dry, exposed areas. Infected fruits and branches arising in the axils of the infected leaves are present on this specimen.



30



31





commonly found in dry, exposed areas or on newly infected branches in cool, moist, shaded regions. The heavy, compact, thickly-branched form is commonly found on older branches in cool, moist, shaded areas. As the loose form is common to both areas, this would indicate that it is an early stage in the development of heavier brooms and that its full development is restricted by environmental factors present in exposed areas.

Young witches' brooms produce slender, smooth-barked branches. In older brooms, the new branches are also slender and smooth-barked, but older branches of the brooms are swollen and have roughened bark which splits and eventually is shed. At the nodes or forks of branches where initial infection occurred, the swelling is very noticeable and the bark is cracked and shedding (Figure 33, page 69). Older infections are readily recognizable in the field by the presence of these older brooms which have a curved central branch with numerous short side branches clustered around it (Figure 32, page 69). The angle of branching for these side branches is reduced compared to that on normal branches. With this feature and the retention of the dead, brown and black leaves on the branches, infections stand out against the healthy green-leaved normal branches.

In infected stems the pathogen is located principally within the phelloderm and cortex regions. The fungus is extremely difficult to observe as it is

Figure 32. Close-up of large witches' broom in Figure 30 showing the curved central branch and the clustering of side branches due to a lessening of the angle of branching.

Figure 33. Close-up of branch to show the roughening and splitting of the bark and the swelling of the branch in the older portion of the infection.



32



33





intercellular and greatly compressed between the host cell walls. It is most noticeable where it crosses between the cells of the fibre bundles in the cortex (Figure 10, page 55). Recently infected stems show little disruption from the presence of the fungus, but in older infections hypertrophy and hyperplasia of the host cells occur.

The total effect of *Apiosporina collinsii* on *Amelanchier* species is not as great as that of *Apiosporina morbosa* on *Prunus* species. I believe that there are three possible reasons for this: (i) the amount of internal mycelium produced by *Apiosporina collinsii* may be less than that produced by *Apiosporina morbosa*; (ii) the internal mycelium of *Apiosporina morbosa* is restricted to the stem, whereas the mycelium of *Apiosporina collinsii* spreads through the stem into the foliar regions, and (iii) *Amelanchier* appears able to restrict the growth of its pathogen to a greater degree than *Prunus*.

In cross sections of uninfected *Amelanchier* stems, the arrangement of tissues is: cork (phellem), phellogen, phelloderm, cortex with bundles of thick-walled fibres, phloem, cambium, xylem (vessels and tracheids) and a pith. The cork consists of a number of layers of cells with progressively thicker cell walls toward the centre of the stem and lumina containing golden to brown substances that are likely resinous or tanniniferous in nature. The xylem consists of annual rings composed of spring wood (large vessels, some tracheids) and summer wood (mainly





tracheids). Rays are typically biseriate, but uniseriate and multiseriate rays are found. The parenchyma cells of the ray are thinner-walled and larger in the phloem and cortex regions than in the xylem.

Infection by the fungus causes hypertrophy and hyperplasia in the cortex region of the stem. In the presence of the fungus, parenchyma cells of the phelloderm, cortex or rays are stimulated to form a cork cambium. The first cells produced by this cambium are large and parenchymatous in nature. With successive divisions the cells produced become thicker-walled, smaller and have golden to dark brown lumina. The end result is the production of rings of cork around infected areas (Figure 34, page 73).

If the fungus reaches the vascular cambium in the region of a ray within the current years growth, the cambium is induced to produce cork cells rather than xylem or phloem. Next to the cork cells, the cambium produces parenchymatous ray cells, then wood consisting entirely of small, thick-walled tracheids. Within the same annual ring, wood consisting of vessels and tracheids is produced indicating that the cambium is not affected by the fungus in these areas. It has been noticed in regions of light infection, that the vascular cambium becomes re-established after the fungus has been walled-off. When this occurs, the cambium goes on to produce normal xylem and phloem in subsequent years. This accounts for the occurrence of occasional islands of cork within older regions of the wood.



In portions of annual rings that are heavily infected, the wood consists mainly of tracheids with few vessels being produced. In these areas the annual rings are narrower than in uninfected regions within the same annual ring. The difference in size is due to the lesser number of vessels.

When older, previously formed wood is infected, the production of a cork cambium does not occur. Instead, the ray cells and adjoining wood cells become filled with resinous or tanniniferous substances (Figure 35, page 73). The effects of the fungus have been observed in eleven year old wood and in the primary and secondary xylem and pith of many younger stems.

It is only in regions where ray cambium is affected by the fungus that abnormally large rays occur. In the cortex, rays remain normal in size (usually bi-seriate) unless directly infected by the fungus or induced to divide by the near presence of the fungus.

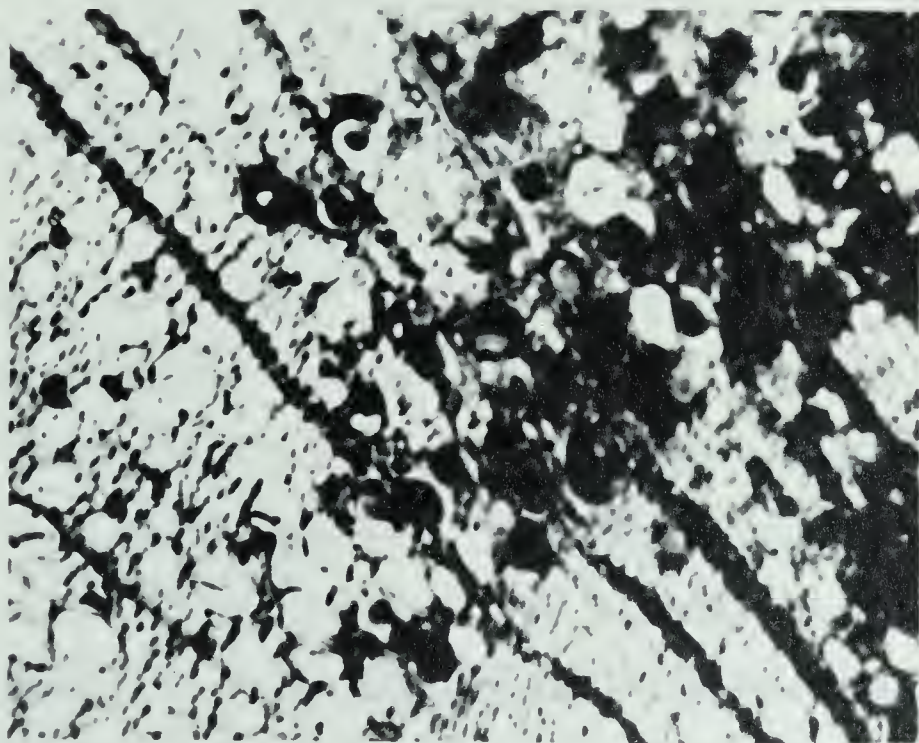
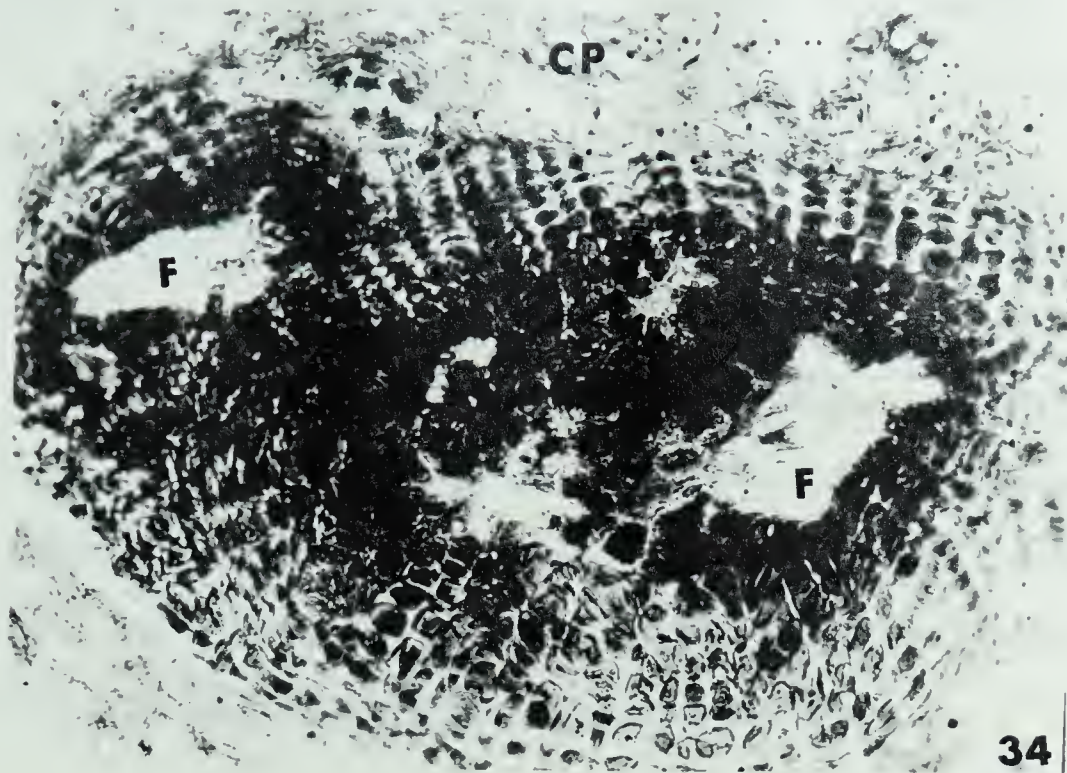
An outstanding feature of the disease caused by *Apiosporina collinsii* in *Amelanchier* is the restriction of brooms to one or two branches. The whole shrub or tree is never infected unless it is a young sapling. Infected saplings which were one to two feet in height were found in heavy mixed woods near Hastings Lake. In most cases all leaves on the stem were infected.

In older shrubs, the host is able to successfully restrict the amount of infection by the production

Figure 34. (X100) Cork patches in the cortex of an infected stem. (CP) cortex parenchyma (F) fibres

Figure 35. (X90) Infected xylem showing vessels, tracheids and ray cells filled with resiniferous or tanniniferous substances.









of cork in infected regions of the stem. When the stem is examined in longitudinal section, cork regions in the cortex can be traced down the sections only a few centimetres (Figures 36 and 37, page 75). The disappearance of the cork indicates the absence of the fungus from the tissues.

The production of cork in the cortex is responsible for the splitting and shedding of the bark on older, infected stems. As new wood and phloem cells are formed, the areas of cork surrounding the fungus are pushed outward and the resulting pressure causes the bark to split over these areas. The bark and portions of the cortex between the bark and new cork patches are sloughed off.

#### b) Effects on the Leaves and Fruits

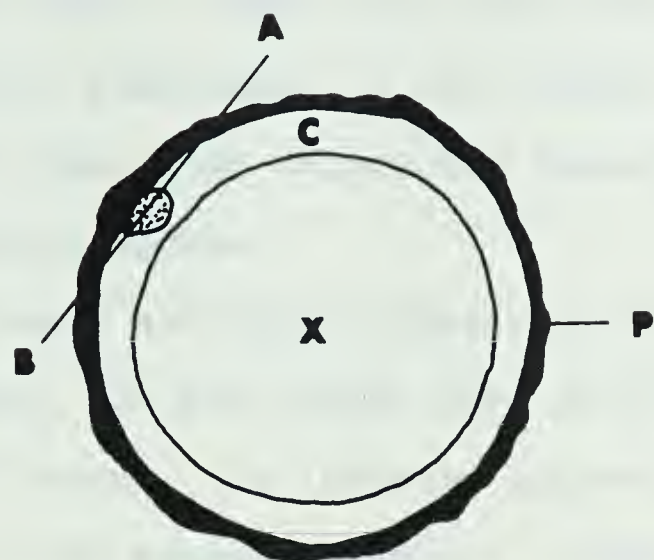
It is on the underside of the leaves that the fungus develops to the fullest for it is here that the production of the conidial, subicular and pseudothecial stages occurs.

Infected leaves are killed by the fungus and may remain on the stem for a number of years. In young brooms the leaves are normal in size, but with each successive crop the leaves become smaller. New leaves are produced on branches arising in the axils of old infected leaves (Figure 31, page 67). The internodes of these new branches become increasingly shorter each year until whorls of leaves are produced at their tips.

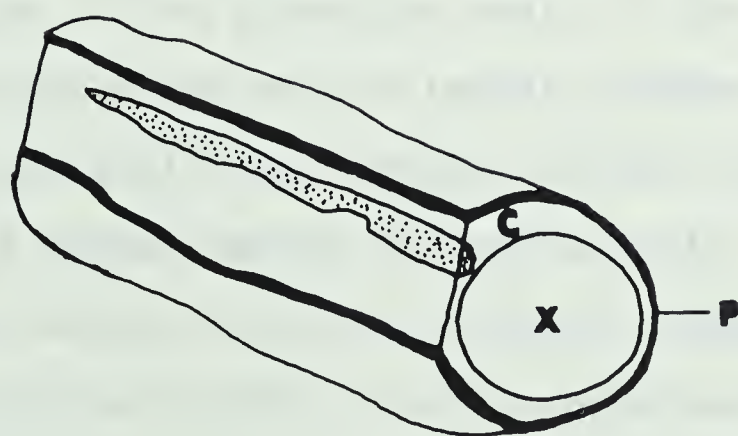
The effect of the fungus on the petiole bases is

Figure 36. (X5.0) Diagram of a cross section of an infected stem showing a patch of cork (stippled) in the cortex. (C) cortex (P) periderm (X) xylem (line AB) position of tangential cut

Figure 37. (X2.5) Diagram of an infected stem cut tangentially to show the tapering of a cork area (stippled) in the cortex. This feature is responsible for the walling-off of the fungus.



36



37



responsible for the leaves remaining in place year after year. In cross sections of infected bases, areas of cork can be found surrounding the leaf traces and occasionally in the parenchyma areas. These cork cells have resiniferous or tanniniferous substances in the lumina. Infected petiole bases are wider than uninfected bases and encircle the stem to a greater degree.

Hyperplasia and hypertrophy are not evident in the infected leaves, but the fungus does have a disrupting influence on the tissues. In infected leaves the mycelium is found mainly in the spongy mesophyll, among the collenchyma, fibres (Figure 9, page 55) and vascular tissues of the veins and, to a lesser degree, in the palisade mesophyll. The fungus pushes apart the mesophyll and lower epidermal cells by completely surrounding them. The host cells are killed and their contents disorganized. The death of these cells results in the premature death of the leaf. Soon after the undersurface of the leaves becomes covered by conidiophores and subicular hyphae, the leaf margins curl under and the leaves become dry and brittle. With the drying of the leaves, the upper surface changes in color from green through yellow, rusty orange and finally brown.

The fungus remains intercellular in the leaf. Haustoria have not been found in the host cells.

As well as being present in the stems, leaves and petioles of the host, the fungus can also be found in





the bud scales, flowers and fruits. Flowers are infected before they open and mycelium can be found in the receptacles and sepals just beneath the epidermis. When the receptacles are infected they fail to develop normally, but remain on the branches as slightly enlarged, shrivelled receptacles (Figure 31, page 67). They have the coloring of mature fruits indicating that pollination and fertilization occurred. While the mycelium often emerges to produce conidiophores, ascocarps have not been found on the fruits.

## B. Notes on the Biology of *Apiosporina morbosa* (Schw.) v. Arx.

### 1. The Ontogeny of the Fungus

The internal mycelium of *Apiosporina morbosa* is confined to the stems of *Prunus* species. The fine, hyaline hyphae can be found in the wood and cortex of the stems. The mycelium is intercellular and lacks haustoria.

When the knot, which is the symptom of the disease, forms, the bark ruptures and exposes the wood and mycelium. On these exposed surfaces numerous olive-brown conidiophores composed of cylindric, smooth-walled cells are produced by the fungus. The conidiophores form smooth-walled, lemon to irregularly-shaped, olive-brown, single-celled conidiospores. Dark brown to black pseudothecia develop amongst the conidiophores on the surface of pseudo-



parenchymatous hyphae which cover the exposed wood and cortex. The developing pseudothecia eventually cover the surface of the knot and the conidiophores are shed.

The centrum of the mature pseudothecium is comprised of pseudoparaphyses and short-stalked, bitunicate, cylindrical asci. The asci contain eight, two-celled, hyaline ascospores which are ovoid in shape with the apical cell being much larger than the basal cell. The spores are arranged in the ascus one above the other with some overlapping. The larger cell is always toward the apex of the ascus. During the development of the pseudothecia, the underlying, pseudoparenchymatous hyphae become thick-walled and carbonaceous in nature to form a stroma over the surface of the knot.

## 2. The Effect of *Apiosporina morbosa* on *Prunus*

The presence of the fungus in the stem causes a great deal of hypertrophy and hyperplasia of the host cells. The result is the production of a large knot, one to several inches in length which may increase the diameter of the stem several times. The knot is composed chiefly of wood and cortical elements with the mycelium of the fungus scattered through it. As the knot increases in size, it eventually ruptures the bark of the stem and causes this layer to be shed. The exposed surface of the knot is at first covered by a pseudoparenchymatous hyphal layer, then conidiophores and finally a carbonaceous stroma bearing pseudothecia.



Large amounts of resinous compounds are produced by the infected host cells beneath the stroma and often granules of resin are found in the fissures of the knots.

### C. Geographical Distribution

Present knowledge indicates that *Apiosporina collinsii* and *Apiosporina morbosa* occur only in North America. In general, both species appear to be as widely distributed as their hosts.

#### 1. *Apiosporina collinsii*

The distribution of specimens of *Apiosporina collinsii* examined is shown in Figure 38 (page 81). This figure is based on only those specimens for which the data supplied was sufficient enough to allow exact location of the collection area on the map.

Locally, it is found most abundantly in the parkland regions of Alberta. Within this region, infections are heavier when the host occurs under cool, moist, shaded conditions and lighter in dry, exposed, sunny areas. The number of infected plants is found to diminish toward the foothills and none has been found in the Rocky Mountains around Banff and Jasper.

#### 2. *Apiosporina morbosa*

*Apiosporina morbosa* has been reported from the







following regions:

<u>Canada</u>	<u>United States</u>
Alberta	Colorado
British Columbia	Idaho
Manitoba	Iowa
Newfoundland	Kansas
Nova Scotia	Maine
Ontario	Massachusetts
Quebec	Michigan
Saskatchewan	New York
	North Carolina
	Ohio
	Washington
	Wisconsin

As only a small number of collections of *Apiosporina morbosa* were available for examination, the distribution list appears short. It is likely that this fungus is much more widely spread since it infects a wide range of *Prunus* species in North America.

Figure 38. Distribution of *Apiosporina collinsii*.





## DISCUSSION

A. Life History of *Apiosporina collinsii*

Although the life history of *Apiosporina collinsii* has been reported on by von Höhnelt (1910), Sartoris and Kauffman (1925) and Sprague and Heald (1927), lack of information or misinterpretations of various aspects of its development have led to confusion concerning the classification of this species. The first part of this discussion deals with the biology of *Apiosporina collinsii* noting new information or information at variance with previous reports.

The development of this fungus begins with the germination of an ascospore or conidiospore in bark fissures or leaf or bud axils of the host *Amelanchier*. Sprague and Heald (1927) attribute the initiation of infection mainly to ascospores. However, as I found both types of spores present in the air and both to germinate readily under moist conditions, I consider that either spore type is capable of infecting the host.

Von Höhnelt and Sartoris and Kauffman state that this fungus is a superficial leaf parasite in which the mycelium enters the leaf tissues only after development has begun on the undersurface of the leaf. My findings oppose this view and agree with those of Sprague and Heald who report the presence of the mycelium in the stem before it





reaches the foliar and floral parts.

Sprague and Heald emphasize that the mycelium is located in the phelloderm of young stems and spreads into the xylem and cortex in older infected stems. My observations agree with theirs, but it should be stressed that the mycelium is very difficult to observe in cross sections of infected, older stems. In this material it is best located in the intercellular spaces of the fibre bundles in the cortex. Here the granular nature of the cytoplasm contrasts with the thick walls of the fibres, whereas in other portions of the stem, the mycelium is greatly compressed between the host cell walls and blends with the contents of these cells. In young infected stems, the mycelium is most easily seen by macerating the material with KOH, then squashing it and staining with cotton blue in lactophenol.

Sprague and Heald state that the fungus has little effect on the host cells in the stem except for some disturbance of the cork cambium. They say "it [the cork cambium] tries to recover by building a new cork cambium inside the fungus zone, thus slowly forcing the parasite out of action." They also explain the roughening of the bark in older infected parts by this feature.

My studies showed that this occurs in some cases and also that the cork cambium alone is not responsible for all the disruption of the host tissues in infected



stems. Hyperplasia and hypertrophy of the host cortical cells also takes place in the vicinity of the fungus. Patches of cork develop in the cortex isolating the fungus from uninfected cells. These patches arise by the division of the host cells and the formation of adventitious cork cambia in the cortex. If the fungus enters the xylem by way of the ray cells, cork cells do not form. Instead the lumina of the ray cells, tracheids and vessels become plugged with resinous or tanniniferous substances. The occurrence of these substances in the cells of this region could account for the "dead charred cells" that Sprague and Heald describe in the infected stems. I could not see the actual mycelium in the infected xylem although its effects were clearly visible. It is possible that the mycelium disintegrates in these regions as Koch (1935) reports for *Apiosporina morbosa* mycelium in older portions of the knots formed in the stems of *Prunus* species.

Sprague and Heald state that the mycelium of the fungus becomes more septate in older portions of infected stems compared to younger portions. Although I have not found this feature to be as striking as they suggest, some variation in the amount of septation between older and younger parts of the mycelium would be expected as this is a common feature in many fungi.

None of the previous workers have tried to explain the restriction of infection to one or a few branches on a shrub. I believe the host is able to restrict the spread





of the mycelium by the production of cork cells which wall off infected areas. This control of the parasite by the host is aided by the tendency for the fungus to grow apically in the stem. Rarely is evidence found for the presence of the fungus more than a few centimetres below the most swollen part of an infected stem.

The fungus enters the leaves of the host while they are still in the bud stage. The mycelium can be traced from the stem through the petioles and into the leaf lamina. Sprague and Heald remark that the fungus is mainly located in the angles of the papillose cells of the petiole epidermis and beneath the lower epidermis in the dormant leaflets. However, I have found that it is first associated with the vascular tissues. From this position it branches to surround the parenchyma cells of the petiole and the spongy mesophyll cells of the leaf. My observations on the development of the mycelium in the expanded leaf confirm those of Sprague and Heald. The mycelium forms a highly branched network around the cells of the spongy mesophyll and rarely penetrates the palisade mesophyll to any extent. These investigators did not report that the fungus is also abundant among the fibres and collenchyma cells of the midrib.

Sprague and Heald mention that the mycelium often seems attached to the cells of the spongy mesophyll in some fashion, but that they could find no haustorial penetrations. My studies confirm the observation that haustoria are not





formed.

Soon after the leaves emerge in the spring, the fungus penetrates between the lower epidermal cells or through the stomata, to spread over the undersurface of the leaf. A superficial, single layer of multiseptate hyphae is formed which is at first hyaline then olive-brown in color. Sprague and Heald also report the occurrence of this layer, but they emphasize the emergence of the fungus through the stomata. In cross sections of infected leaves it is difficult to find two adjacent lower epidermal cells without a branch of the mycelium passing between them to connect to the surface layer.

While still on the topic of infected leaves, it should be mentioned that Sprague and Heald report that these leaves often fall in early summer or, if they remain on the branch until the following spring, then a second crop of leaves is not produced. I did not find this to be the case in this area. Infected leaves frequently remain on the branches for a number of years and, in such cases, I have often found that small branches are produced in the axils of these leaves.

There is little variation between the findings of von Höhnelt, Sartoris and Kauffman, Sprague and Heald and those of the present study regarding the conidial phase in the life cycle of *Apiosporina collinsii*. The conidiophores are three to five-celled, simple, rarely branched, olive-brown structures arising from bulbous basal cells



produced by the superficial layer of septate hyphae. The conidiospores are single-celled, olive-brown, typically lemon-shaped, but may vary from ovoid to cylindrical to irregular in shape. The spores are produced primarily at the conidiophore apex, but may arise at the apical ends of the other cells. My findings show that the apical cells of the conidiophores are also capable of germination.

Sprague and Heald give the best previous account of variation in conidiospore size and shape, but state that the spores are first produced in chains then singly. I have not seen this feature. They report difficulty in getting the spores to germinate on agar plates. I found that the spores germinate within 48 hours in distilled water.

While the other workers all describe a fungal-felt or mat on the undersurface of the leaves, none of them suggest a function for this stage of the life cycle other than stating that the ascocarps are embedded in this subiculum. On the basis of my observations, I consider that the subiculum is instrumental in the formation of the ascostromata in which the asci and ascospores eventually arise. The ascostromata are perithecioid in shape, hence, they are referred to as pseudothecia.

The mature pseudothecia are black, globose, short-stalked structures, occasionally laterally fused. The presence of a short stalk is noted only by von Höhnelt, although the photographs of Sartoris and Kauffman and Sprague and Heald illustrate this feature. The mature





pseudothecial wall is composed of two zones: an inner, hyaline zone comprised of three to four layers of flattened cells; and an outer dark brown to black zone made up of three to four rows of isodiametric, thick-walled cells. This zonation has not been reported previously.

Although former investigators report the presence of blunt hairs or bristles on the pseudothecial wall, my findings show that this is not a constant feature. Wall appendages occur only when a subicular branch fails to become incorporated into the hyphal knot which will develop into a pseudothecium. For this reason the presence or absence of appendages cannot be considered a diagnostic character.

The centrum of the pseudothecium undergoes a number of developmental stages before the production of mature ascospores. These stages will be discussed in some detail since, in the schemes of classification now in use, the development and characteristic features of the centrum are important to the final placement of the organism within taxonomic categories.

Von Höhnelt describes the pseudothecium as containing simple paraphyses which over top the thick-walled, short-stalked, cylindrical asci in which eight two-celled, hyaline ascospores are formed.

Sartoris and Kauffman describe the following development for the pseudothecium and its centrum.





"The coils of mycelium which are formed during the first part of July are the beginnings of the perithecia. In the centre of each coil are one or two slightly differentiated hyphae which contain fine, granular protoplasts. These stain deep blue with Haidenhain's haematoxylin. They are enveloped by a number of vegetative hyphae which become closely packed to form the perithecial wall. The central cells continue to divide until many cells are formed, always in a uninucleate condition. When the wall of the perithecium is completely formed, the central coil sends out a fan-shaped group of hyphae, which grow until they reach the top of the perithecium."

"Prior to the formation of the fan-shaped group of hyphae, conditions arise in certain cells which are suggestive of the manner in which a binucleate cell is formed in the young ascus. Some terminal cells become binucleate by a nuclear division which is not followed by wall formation between the daughter nuclei. Then cell walls are laid down between the homologous members of each pair of granddaughter nuclei, thus forming three cells, the central one of which is binucleate. Then the two elements of the binucleate cell fuse. This, then, is the fusion which initiates the growth of the ascus. Probably before this fusion takes place, the terminal, uninucleate cell will have grown into a slender hypha which is one of the fan



-shaped group of hyphae. The ascus now increases in length and breadth, while the slender hypha terminating it gradually degenerates and is finally cut off at the cross-wall."

They further describe the formation of the ascospores by free-cell division, stating that the mature spore is two-celled with a nucleus in each cell. Sprague and Heald describe the centrum of the pseudothecium to be composed of thread-like paraphyses and cylindrical asci which are long "bottle-shaped" and contain eight, two-celled, hyaline spores.

The following is a summary of my observations on the development of the centrum of *Apiosporina collinsii*. The centrum is first composed of pseudoparenchymatous hyphae after which a fan-shaped group of hyphae, produced by the inner wall, appear at the apex of the centrum. These hyphae grow downward and fuse with cells in the base of the centrum or with each other. These hyphae may be correctly termed pseudoparaphyses as defined by Luttrell (1965). The hyphal fusions give rise to binucleate cells in the basal region of the centrum. These cells are the ascal initials which enlarge and grow upward becoming mature asci containing eight, two-celled, hyaline ascospores. The asci are bitunicate.

Pseudoparaphyses were not described in the papers published by von Höhnelt, Sprague and Heald and Sartoris and





Kauffman. The occurrence of these structures must be emphasized as they are a taxonomically important feature. A second important character is the bitunicate nature of the ascus which was also omitted from the reports of these investigators.

The description of the development of the pseudothecium given by Sartoris and Kauffman is confusing. From my studies, and from information reported in the literature on the development of other ascostromatic Ascomycetes, the description of Sartoris and Kauffman appears to contain many misinterpretations of the events in the centrum development. They speak of the perithecium [pseudothecium of this thesis] arising from coils of hyphae with two differentiated cells in the centre. This description reflects the old idea that all perithecia are derived from archicarps. Had they recognized the perithecium to be a stromatic structure, their views might have been different.

The following is an account of the three main types of ascocarp initiation known to occur in Pyrenomycete fungi as given by Munk (1953).

- a) "Two apparently similar hyphal endings coil spirally round each other, and the plasmatic fusion takes place somewhere in the coil (*Sordaria*, *Xylaria*). This modus is also known in Plectascales, e.g. *Penicillium*. There is no visible differentiation in male and female organs."





b) "A pronounced differentiation in male and female organs: the female cell ("archicarp") is immersed in a stroma (*Polystigma*) or surrounded by a small, well delimited, sometimes superficially seated tissue of the structure textura globulosa-prismatica (*Neurospora*). The copulation seems to be carried out by very small, conidium-like cells (spermatia), which are received by a thin, prominent hypha (trichogyne) from the archicarp. Both of these types of development are found in the ascohymenial Pyrenomycetes."

c) "A hypha undergoes three-dimensional septation; in the resulting parenchymatic lumps of cells the sexual processes (probably) occur (*Sporormia*). This type of development is found among the Ascoloculares."

It is my opinion that the coils which Sartoris and Kauffman describe are in actual fact the pseudoparenchymatous centrum found in the early stage of development of a pseudothecium. I have never observed the "two slightly differentiated hyphae" of their report. The initiation of the pseudothecium of *Apiosporina collinsii* corresponds to Munk's third type (c). The fan-shaped group of hyphae which Sartoris and Kauffman describe as developing from the coil and growing upward are probably the pseudoparaphyses. Their photograph of these hyphae is identical with



my Figure 15 (page 58). Sartoris and Kauffman appear to have wrongly interpreted the nature of these hyphae, their direction of growth, the function of these hyphae, and the time of their development. Their statement: "Some terminal cells become binucleate by a nuclear division which is not followed by wall formation between the daughter nuclei."; is difficult to understand, as they do not give any indication of the origin or location of these cells.

Until recently, the pseudoparaphyses were considered to function only in the formation of the locule within the pseudothecium in which the asci would ultimately arise. Gordon (1966), studying members of the Hypodermataceae, reports two basic types and one intermediate type of centrum ontogeny for this group. The type he designates as Type I, based on characteristics of *Lophodermium juniperinum* follows very closely the development occurring in *Apiosporina collinsii*. Gordon's description of Type I is as follows:

"Squashing the centrum and the basal pseudo-parenchymatous tissue reveals that the basally septate portions of the pseudoparaphyses are uninucleate. Anastomoses are numerous between these portions. The nucleus from one anastomosing cell migrates into another. Since the same cell may anastomose with two or more cells, the two nuclei





resulting from a previous anastomosis may migrate into yet another cell that already has one or two nuclei. Therefore, any of the basal pseudoparaphysate cells may be completely devoid of nuclei or contain one to four of them.

Many of the cells containing at least two nuclei begin to enlarge. This expansion is usually lateral to the original axis, not isodiametric. When the ascal cell has become approximately twice its original size, a nucleus larger than any of the previous nuclei can be seen. At this point, the ascal cell is growing upward. As elongation continues, the young uninucleate ascus pushes aside the lower cells of other pseudoparaphyses."

In *Lophodermium juniperinum*, the pseudoparaphyses are attached to the top of the centrum only in the early stages of development according to Gordon. The apical portions of these pseudoparaphyses become the inner part of the clypeus and one cell of each disintegrates causing these hyphae to appear paraphysate in later stages of development. The only major difference occurring between the mode of centrum development that Gordon reports and the development of the centrum in *Apiosporina collinsii* is that the pseudoparaphyses remain attached to the top of the centrum in this fungus.

During early stages of ascal development in *Apio-*





*sporina collinsii*, the pseudoparaphyses can still be seen attached to the young asci. This feature may explain what Sartoris and Kauffman saw when they describe the development of the ascus within a hyphal strand, the tip of which degenerates when the ascus begins to enlarge. However, their description of ascal development appears to have been incorrectly determined.

The pseudoparaphyses of *Apiosporina collinsii* do disintegrate ultimately, but not until just prior to the opening of the pseudothecium and the discharge of its ascospores. This stage of development is described in detail in the observations section of this thesis (page 63).

Von Höhnelt (1910) states that the ascocarps open by means of ostioles and Sprague and Heald (1927) report the ascocarps to be "apparently ostiolate". Sartoris and Kauffman (1925) say that von Höhnelt's generic description requires emending as the ascocarps are "not with ostioles". Although they recognize that true ostioles are lacking, they do not describe the type of opening that does occur.

None of these authors describe the method of spore release or the type of spore dissemination. I found that following the opening of the ascocarp, an ascus extends through the pore, the outer wall splits to allow extension of the inner wall and the ascospores are forcefully ejected one after the other from the ascus. The released spores are then wind disseminated.

Sartoris and Kauffman report the presence of



pycnidia on the upper surface of infected leaves in the fall. They do not suggest a function for these structures. I have occasionally found pycnidia to be present, but have been unable to determine whether or not these structures actually represent a stage in the life cycle of *Apiosporina collinsii* or function in the development of this fungus. As they occur infrequently it is likely that they are produced by another organism which is parasitic on the same host, or if the pycnidia do represent a stage in the life cycle of *Apiosporina collinsii* their occasional appearance would indicate that these structures are now functionless. The discussion of these structures by Sartoris and Kauffman supports this view.

Before closing this portion of the discussion dealing with the life history of *Apiosporina collinsii* I wish to discuss the question of whether or not a stroma is present during the development of this fungus.

Von Höhnelt reports the presence of a dark brown hypostroma produced by the fungus on the underside of the leaf. Sartoris and Kauffman state that due to the numerous perithecia on the undersurface of the leaves by late August, the surface becomes covered by a black stroma-like layer mixed with perithecia. Sprague and Heald (1927) state that stromata and sclerotia occur on the undersurface of infected leaves.

Much of the confusion over the presence or absence of a stroma is due to the wide range of structures to which





this term has been applied in the past. Using the term stroma, as defined in the glossary of this thesis, it can be stated that *Apiosporina collinsii* does not produce a stroma at anytime during its life cycle. The single layer of mycelium which covers the undersurface of the leaves and gives rise to the conidiophores and subiculum could never be considered a stroma. Although it tends to unite all the pseudothecia and eventually turns dark olive-brown in color, its development is not extensive enough to be considered as a stroma. I have not found any structures that could be termed sclerotia. It is possible that the structures referred to as sclerotia by Sprague and Heald are in actual fact the hyphal knots appearing as the earliest stage in the development of the pseudothecia.

#### B. Taxonomy of the Genus *Apiosporina*

Before the classification of *Apiosporina collinsii* can be decided, it is necessary to revise the species description to include new information on the pseudothecium and on centrum development. Considerable importance is attached to such information in the system of classification developing today for the pyrenomycete-like fungi. It is also necessary to emend the original generic description to include the species *Apiosporina morbosa*.





The following species description for *Apiosporina collinsii* includes the findings of previous workers and new information presented in this thesis.

*Apiosporina collinsii* (Schw.) von Höhnelt

Mycelium hyaline in stems, buds, leaves, flowers and fruits, emerging to form a single layer of multi-septate hyphae with cells  $3.2 - 4.8 \times 6.4 - 11.2 \mu$  over the undersurface of the infected leaves. This layer, at first hyaline, later olive-brown, gives rise to conidiophores and subicular hyphae. Conidiophores mostly simple, with a bulbous basal cell  $8 - 9.6 \mu$  in diameter, and three to five, smooth-walled, cylindrical, olive-brown cells  $3.2 - 4.8 \times 11.2 - 24.0 \mu$ . Conidiospores single-celled, olive-brown, lemon to irregular-shaped  $6.2 - 9.3 \times 7.8 - 21.7 \mu$ . A terminal conidiophore cell may act as a conidiospore. Subiculum hyphae frequently branched, dark olive-brown. Pseudothecia arise in the subiculum, crowded, occasionally laterally fused, short-stalked, globose,  $150 - 250 \mu$  in diameter, opening by an uneven pore, walls two-zoned: outer wall three to four layers of thick-walled, isodiametric, carbonaceous cells; inner wall three to four layers of hyaline, flattened cells. Asci bitunicate, short-stalked, cylindrical,  $6.4 - 8.0 \times 56 - 72 \mu$ , containing eight ascospores and associated with pseudoparaphyses.



Ascospores ovoid, one-septate toward the pointed end, uni-seriate to slightly overlapping, hyaline,  $4.0 - 6.0 \times 12 - 15 \mu$ .

Systematic parasite of *Amelanchier* species in North America, causing the formation of witches' brooms by the host. The underside of the leaves is the prime site of infection.

The features presented in this description that are of importance to the classification of this organism are: the pseudothecia arising from the subiculum, centra with pseudoparaphyses, and bitunicate asci containing eight, two-celled, hyaline ascospores.

The most recent description of *Apiosporina collinsii* given by Müller and von Arx (1962) requires some emendation and comment. They state that a stroma develops on the underside of fallen *Amelanchier* leaves and gives rise to the fruiting structures. My observations show that the infected leaves do not fall and the fruiting structures (pseudothecia) arise from a subiculum. This species lacks any structure which could be considered a stroma. Müller and von Arx report the occurrence of a subiculum, but they say that it forms after the initiation of the ascocarps. I have found the subiculum to arise first and take part in the formation of the ascocarps. These workers also report that the ascospores are hyaline at first and then become bright brown or yellow at maturity. In my material only





hyaline ascospores occurred.

In 1954, von Arx placed *Dibotryon morbosa* in the genus *Apiosporina* and it is so classified by Müller and von Arx (1962). In Table I (page 101), a comparison of the characters of *Apiosporina collinsii* and *Apiosporina morbosa* is presented based on descriptions and reports of Farlow (1876), Koch (1935), von Arx (1954) and my investigations. From this table, it can be seen that von Arx was justified in placing these species in the one genus. The only structural difference between them is that *Apiosporina morbosa* produces a stroma while *Apiosporina collinsii* produces a subiculum. As the stroma and subiculum are both vegetative structures which give rise to the pseudothecia, I believe that this difference is not significant. In addition, both species parasitize members of the Rosaceae and occur only in North America with a distribution almost as wide as that of their hosts. It seems that these species form a natural unit when placed together.

Material of *Apiosporina harunganae* was unavailable for examination. However, judged on the basis of Hansford's (1947) original description, this species does not belong in the genus *Apiosporina*. Table II (page 102) presents a comparison of the features of Hansford's species with those of the genus *Apiosporina* as emended.

Farr (1963) in her study of *Dimeriella* notes that the possession of ellipsoidal asci, ascospores with a median septum and an apparent lack of a conidial stage by





TABLE I

Comparison of *Apiosporina collinsii*  
with *Apiosporina morbosa*

<i>Apiosporina collinsii</i>	<i>Apiosporina morbosa</i>
Parasitic on <i>Amelanchier</i>	Parasitic on <i>Prunus</i>
Reproductive structures on underside of leaf	Reproductive structures on surface of stem galls
Pseudothecia in a subiculum	Pseudothecia borne on the surface of a stroma
Pseudothecia spherical, crowded, cover entire undersurface of leaf	Pseudothecia spherical, crowded, cover entire surface of stroma
Filamentous pseudo- paraphyses present	Filamentous pseudo- paraphyses present
Asci cylindrical, bitunicate, short-stalked, 6.4 - 8.0 x 56.0 - 72.0 $\mu$	Asci cylindrical, bitunicate, short-stalked, 12 - 15 x 58 - 74 $\mu$
Ascospores hyaline, ovoid, two-celled, septum toward pointed end, 4 - 6 x 12 - 15 $\mu$	Ascospores hyaline, ovoid, two-celled, septum toward pointed end, 5 - 6 x 15 - 18 $\mu$
Conidiospores <i>Cladosporium</i> -like, olive-brown, single-celled, mainly lemon-shaped	Conidiospores <i>Cladosporium</i> -like, olive-brown, single-celled, mainly lemon-shaped



TABLE II

Comparison of the characters of the species *Apiosporina harunganae* with those of the genus *Apiosporina*

<i>Apiosporina harunganae</i>	<i>Apiosporina</i>
Mycelium pale brown, superficial, with hyphopodia	Mycelium hyaline, systemic
Perithecia loose, irregularly dispersed	Pseudothecia crowded, cover entire surface of a stroma or subiculum
Perithecium wall consists of an outer single layer of blackened cells and an inner wall of indistinct hyaline cells	Pseudothecium wall two-zoned, outer zone 3-4 cell layers brown-black isodiametric cells, inner zone 3-4 layers flattened hyaline cells
Wall with scattered, pointed setae	Wall without regular appendages
Asci short-stalked, ellipsoid	Asci short-stalked, cylindrical
Aparaphysate	Pseudoparaphysate
Ascospores smooth, clavate, median septate, pale olive when mature	Ascospores smooth, ovoid, septate toward narrow end, hyaline when mature
No conidial stage reported	<i>Cladosporium</i> -like conidial stage



*Apiosporina harunganae* suggests resemblance to *Dimeriella balsamicola*. She also states that Hansford's species departs from the generic concept of *Apiosporina*. It seems reasonably certain that *Apiosporina harunganae* does not belong in the genus *Apiosporina*, but only the study of type material can settle this question.

Before classifying the species of *Apiosporina* it is necessary to decide which features of the genus should be stressed in order to show relationship with other genera. Let us consider available features and their value for this purpose.

(1) The morphology of the ascocarp, a feature given prime importance in past classification schemes such as Lindau's (1897), has been used. However, this is a vegetative structure in the case of *Apiosporina* and subject to variation. Sprague and Heald (1925) reported finding the perfect stage of *Apiosporina collinsii* rarely in the Pullman, Washington area, while in Alberta the ascocarp stage is abundant. In fact nearly every infected leaf becomes covered with pseudothecia in the fall. It is likely that some environmental factor in their area limited the development of this fungus to the imperfect stage.

Wehmeyer (1961) in his monograph of *Pleospora* and its segregates, places little emphasis on the details of the ascostomata (pseudothecia) as they are primarily





vegetative structures and may vary greatly in size and thickness of wall. He also remarks that the presence of tomentum or setae on the ascostromata must also be used with care, since such ornamentation is often variable within species and is frequently broken off or shed with age. Evidence in support of this latter point has been found in the present study. *Apiosporina collinsii* is often described as having blunt bristles on the pseudothecia while *Apiosporina morbosa* is described as having glabrous pseudothecia. I found that the bristles of the former species occur only when occasional branches of the subiculum fail to become incorporated into the knot which becomes the pseudothecium. Furthermore, the fact that both species have similar ascocarps which are derived from different structures lends additional support to the idea that the pseudothecial characters should not be considered of prime importance in the taxonomy of these fungi.

(2) The asci are the same in both species, varying only slightly in size which is likely correlated with the slight variation in size of the ascospores between the two species. The asci are bitunicate, with a small "dimple" in the apical wall, cylindric and short-stalked. The bitunicate nature of the ascus when correlated with the occurrence of pseudothecia is the important feature for the placement of this genus in the subclass Loculoascomycetes (sensu Luttrell, 1955).

(3) The occurrence of pseudoparaphyses in the two



species of this genus is of value for placement of the genus at the ordinal level. These hyphae do not serve to show relationship with particular species, however, as little variation occurs in the structure of pseudoparaphyses other than size. The presence of pseudoparaphyses places *Apiosporina* in the order Pleosporales of Luttrell (1955).

(4) Since none of the above characters will serve to show the relationship of *Apiosporina* to other genera, the only character left is the nature of the ascospores. In this genus these spores are ovoid, two-celled with the septum toward the lower end, and hyaline. Wehmeyer (1961) considers the morphology and development of the ascospores in *Pleospora* to be of prime importance for recognition of species. He states "nearly every species has a characteristic spore form, sequence of septation, and intensity of coloration". It is my opinion that the characters of the ascospore should be used to define the genus *Apiosporina* and to indicate relationships between this genus and other genera. I feel that too much emphasis should not be placed on spore color as this feature is often variable and, therefore, of little use in determining generic limits.

Farr (1963) cites investigators who believe that ascospore color is unsuitable for delimiting genera and species of certain pyrenomycete groups. This point is stressed in her studies of the genera *Dimeriella*, *Wentio- myces*, *Episphaerella* and *Epipolaeum* (1965) which she describes as being similar in every character except spore





color and habit. She says "habit or biology is considered a more reliable diagnostic character than ascospore color in classification of these genera". The unsuitability of the character of ascospore color is also stressed by Müller and von Arx (1962).

On the basis of these studies I would set the generic limits of *Apiosporina* in the following manner:

*Apiosporina* von Höhnelt

Conidial stage consisting of olive-brown, simple or occasionally branched conidiophores producing olive-brown, single-celled, lemon to irregular-shaped, *Cladosporium*-like conidiospores. Pseudothecia globose, brown-black, crowded, occasionally laterally fused, seated in a subiculum or on a carbonaceous stroma. Centrum with pseudo-paraphyses and cylindrical, short-stalked, bitunicate asci containing eight, two-celled, ovoid, ascospores. Ascospores septate toward the narrow end. Systemic parasites of Rosaceae.

This genus should include only those species which conform to the characteristics of the ascospores and details of the centrum presented in the above description. Biologically, it is proposed that this genus contain only systemic parasites which will serve to distinguish it from the numerous leaf parasites



having similar centrum development, but a wide range of ascocarp shapes.

As previously stated, the genus *Apiosporina* is placed in the subclass Loculoascomycetes on the basis of the occurrence of bitunicate asci in pseudothecia and in the order Pleosporales because of the pseudoparaphysate centrum. Müller and von Arx (1962) place *Apiosporina* in their family Venturiaceae. In this family they have grouped species which possess ovoid or ellipsoid, two-celled ascospores which are first hyaline, then yellowish, greenish or dark brown. They consider that the ascospore structure is of primary importance and that this family is composed of a group of related species. Munk (1953) supports their opinion.

The species are further related on secondary, structural characteristics and on their biology. The life cycles of *Venturia* and *Apiosporina* are very similar and they have the following features in common: parasitic on members of the Rosaceae; development of conidial stages; ascostromata that contain pseudoparaphyses which gelatinize before spore release; over-winter development of ascospores; unequally septate, two-celled ascospores. However, several differences do occur between these life cycles. *Apiosporina* species are systemic parasites, while *Venturia* species are leaf parasites. Secondly, the ascocarps of *Apiosporina* overwinter on stems or attached leaves, while those of *Venturia* overwinter on fallen leaves. Finally,





although both genera have unequally two-celled ascospores, these spores vary in shape. The upper cell of *Venturia* ascospores is shorter and wider than the lower cell, whereas, in *Apiosporina* the over-all shape of the spores is ovoid, with the septum occurring toward the narrow end giving a very large apical cell and a much smaller basal cell. While this comparison of *Venturia* and *Apiosporina* is very general and does not go into details of size, shape and color of ascocarps and spores, it serves to illustrate that ascospore morphology and general biology of the organisms can be used to show relationship. *Apiosporina* is related to other genera in the Venturiaceae such as, *Gibbera*, *Coleroa* and *Anntenularia* on general features of centrum structure, parasitic growth on leaves and stems and, more specifically, on characters of the ascospores. Because of these relationships, I consider that the genus *Apiosporina* should remain in the Venturiaceae. I would place this family in the order Pleosporales of Luttrell rather than the Pseudosphaeriales where it is classified by Müller and von Arx (1962). I propose classifying the genus *Apiosporina* as follows:

Class - Ascomycetes  
           Subclass - Loculoascomycetes  
             Order - Pleosporales  
               Family - Venturiaceae

This treatment combines features of the classification





schemes being developed by Luttrell and by Müller and von Arx.



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## A P P E N D I C E S





## APPENDIX A

Herbarium collections of *Apiosporina collinsii* examined

## CANADA

Alberta

Cottage Lake	A.W.L. Stewart S386	
	July 4, 1965	ALTA
Crimson Lake	M. Ostafichuk S397	
	1965	ALTA
Devon	A.W.L. Stewart S340	
	June 2, 1964	ALTA
Edmonton	A.W.L. Stewart S359	
	August 12, 1964	ALTA
Edmonton	A.W.L. Stewart S369	
	November 5, 1964	ALTA
Edmonton	A.W.L. Stewart S370	
	February 11, 1965	ALTA
Edmonton	A.W.L. Stewart S389	
	August 20, 1965	ALTA
Edmonton	A.W.L. Stewart S399	
	May 17, 1966	ALTA
Edmonton	A.W.L. Stewart S400	
	May 17, 1966	ALTA
Edmonton	A.W.L. Stewart S401	
	May 17, 1966	ALTA



George Lake	A.W.L. Stewart S346	
	June 12, 1964	ALTA
George Lake	A.W.L. Stewart S347	
	June 12, 1964	ALTA
George Lake	A.W.L. Stewart S348	
	June 12, 1964	ALTA
George Lake	A.W.L. Stewart S350	
	June 12, 1964	ALTA
Hastings Lake	A.W.L. Stewart S374	
	May 11, 1965	ALTA
Hastings Lake	A.W.L. Stewart S375	
	May 11, 1965	ALTA
Hastings Lake	A.W.L. Stewart S376	
	May 11, 1965	ALTA
Hastings Lake	A.W.L. Stewart S377	
	May 11, 1965	ALTA
Muir Lake	A.W.L. Stewart S378	
	May 12, 1965	ALTA
Peace River	P.M. Simmonds	
	2422	DAOM
Priddis	R.A. Owens S396	
	December 27, 1965	ALTA
Spruce Grove	A.W.L. Stewart S372	
	April 30, 1965	ALTA
U of A Botanic Garden	L.L. Kennedy S341	
	June 7, 1964	ALTA



U of A Botanic Garden	A.W.L. Stewart S355	
	August 6, 1964	ALTA
U of A Botanic Garden	A.W.L. Stewart S356	
	August 6, 1964	ALTA
U of A Botanic Garden	A.W.L. Stewart S357	
	August 6, 1964 (2 coll.)	ALTA
U of A Botanic Garden	A.W.L. Stewart S358	
	August 6, 1964	ALTA
U of A Botanic Garden	A.W.L. Stewart S363	
	September 3, 1964	ALTA
Vegreville	M. Shantz & R. Piemeisel 25-491	
	August 26, 1925	NY
Whitecourt-Edson Rd.	A.W.L. Stewart S364	
	September 22, 1964	ALTA
 <u>British Columbia</u>		
Alkali Lake	J.A. Calder	
	93653	DAOM
Barrière	J.A. Calder	
	93650	DAOM
D'Arcy	J.R. Hausbrough 261	
	June 27, 1931	NY
Fort St. James	J.A. Calder	
	55061	DAOM
Kamloops	J.A. Calder	
	55680	DAOM
Owl Creek	J.R. Hausbrough 258	
	June 24, 1930	NY





Pemberton	N.S. Wright 1930	
	September 7, 1946	UBC
Prince George	W.G. Ziller	
	25180	DAOM
Quesnel	N.S. Wright 1513	
	July 1948	UBC
Rosebery	J.R. Hausbrough 259	
	August 2, 1930	NY
Sword Creek	J.A. Calder	
	93651	DAOM
Vernon	V. Krajina 1528	
	August 22, 1950	UBC
Wire Cache	D.C.B. u659	
	June 4, 1950	UBC
150 Mile House	J.R. Hausbrough 260	
	September 14, 1930	NY
 <u>Manitoba</u>		
Brereton Lake	C.G. Riley	
	23235	DAOM
McMunn	R.A. Shoemaker	
	106156	DAOM
Victoria Beach	H. Groh	
	15493	DAOM
Winnipeg	J. Conners	
	35749	DAOM



New Brunswick

Pinder	C.A. Arthurs	
	49184	DAOM
St. Andrews	T. Mounce	
	2065	DAOM

Ontario

Buckham Bay	G.D. Darker	
	90892	DAOM
Dorset	I.L. Conners	
	7304	DAOM
Dryden	R.F. Cain	
	58131	DAOM
Lake Temagami	I.L. Conners	
	1550	DAOM
Lake Temagami		
	35587	DAOM
Lake Temagami		
	35757	DAOM
Lake Temagami	G.E. Thompson	
	86287	DAOM
Lake Temagami	G.D. Darker	
	88782	DAOM
Lake Temagami	G.E. Thompson	
	June 12, 1931	NY
Manitoulin Island	R.F. Cain	
	29608	DAOM





Port Frank	J. Dearness	
	September 1891 (2 coll.)	NY
Spanish River, Algoma	J. Dearness	
	August 1906	NY
 <u>Prince Edward Island</u>		
Crapud	D. Erskine	
	40455	DAOM
Dundee Sta.	A.J. Smith	
	40456	DAOM
Tignish	F.W. Anderson	
	July 25, 1868	NY
 <u>Quebec</u>		
Claude River	A.W. McCallum	
	1782	DAOM
Kamouraska	A. Payette	
	19663	DAOM
La Malbaie	Frère Anseline	
	7717	DAOM
Montreal	J. Brunel	
	May 21, 1932	NY
Mont Tremblant	G.D. Darker	
	89793	DAOM
Nicolet	Rev. Fr. Allyre	
	19755	DAOM



Port Rouge	Frère Anseline	
	62277	DAOM
Ste. Adèle	Frère Anseline	
	13280	DAOM
<u>Saskatchewan</u>		
Indian Head	P.M. Simmonds	
	1403	DAOM
Prince Albert	M. Shantz & R. Piemeisel 25-491	
	August 26, 1965	NY
UNITED STATES OF AMERICA		
<u>California</u>		
Emigrant Gap	H.W. Harkness	
	November 1885 (4 coll.)	NY
	McBride	
	1888	NY
<u>Connecticut</u>		
	W.G. Farlow 84	
	June 1878	NY
	Dr. Thom	
		NY
<u>Idaho</u>		
Bovill	J.R. Weir	
	July 1921	NY



Lake Waha	A.A. & E.E. Heller	
	July 9, 1896 (2 coll.)	NY
Latab Co.	C.V. Piper 248	
	July 6, 1894	NY
Pierce	J.R. Hausbrough 262	
	August 1, 1931	NY
Priest Lake	A.W. Slipp 300-Sta.3	
	July 23, 1939	NY
 <u>Iowa</u>		
Decorah	E.W.D. Holway	
	June 5, 1892	NY
 <u>Massachusetts</u>		
Dartmouth	Ellis: North American Fungi 488	
	W.G. Farlow 405(b)	
	June 1878 (3 coll.)	NY
Magnolia	C.E. Clark	
	July 1883	NY
Magnolia	W.G. Farlow	
	June 18, 1905	NY
Pigeon Cove	A.B. Seymour	
	July 28, 1890	NY
Sharon	A.P.D. Piquet	
	6231	DAOM
Sharon	A.P.D. Piquet	
	August 1903	NY





Wood's Hole	A.B. Seymour	
	6327	DAOM
Wood's Hole	W.G. Farlow	
	June	NY
Wood's Hole	A.B. Seymour	
	June 18, 1900	NY
Wood's Hole	A. Whiting	
	1925	NY
 <u>Michigan</u>		
Marquette	G.H. La Roi S395	
	May 21, 1965	ALTA
 <u>Montana</u>		
Libby	J.R. Weir 24	
	1911	NY
Thompson Falls	D.B. Swingle	
	July 3, 1909	
 <u>New Hampshire</u>		
Randolf	E.B. Marris	
	35588	DAOM
 <u>New Mexico</u>		
Brazos R.	P.C. Standley	
	August 21, 1914	NY



Chama	P.C. Standley 58	
	July 9, 1911	NY
<u>New York</u>		
Albany	C.H. Peck	
	1876	NY
Crane Mtn. Pond	S.J. Smith 36100	
	July 23, 1963	NY
East Cawga Lake	H.D. House	
	August 6, 1923	NY
Essex Co.	S.J. Smith 30685	
	October 6, 1960	NY
Fulton Co.	S.J. Smith 29536	
	Summer 1959	NY
Jamesburg	15	
	July 1889	NY
Panther Lake	W.C. Muenschner	
	B. Brown	
	July 9, 1947	NY
Suffolk Co.	S.J. Smith 29536	
	August 7, 1959	NY
Suffolk Co.	S.J. Smith 31275	
	July 12, 1961	NY
<u>North Carolina</u>		
Leicester	B.B. Higgins	
	June 4, 1909	NY





North Dakota

Cannonball River

J.F. Brenckle

August 21, 1922 (2 coll.)

NY

Johnson Gulley

J.F. Brenckle

June 11, 1922

NY

Ohio

Lancaster

W.A. Kellerman

October 18, 1904

NY

Oregon

Benton Co.

R. Sprague

April 25, 1936

NY

Wallowa Lake

C.L. Shear

August 25, 1899

NY

Pennsylvania

Juniata River

J.T. Bothrock

1887 (2 coll.)

NY

Stone Valley

P.C.R. Orton 61

June 13, 1920

NY

Tobacco Patch Mtn.

W.W. Diehl

June 24, 1934

UBC

Washington

Lucerne

J.R. Weir

August 24, 1916

NY



Onion Crk., Northport	A.W. McCallum	
	268	DAOM
Skamania Co.	W.N. Suksdorf 295	
	August 13, 1886	NY
 <u>West Virginia</u>		
Fayette Co.	L.W. Nuttall 472, 473	
	May 4, 1894 (2 coll.)	NY
Kegley	E.L. Morris	
	July 27, 1900	NY
Nuttallburg	L.W. Nuttall	
	May 1894	NY
 <u>Wisconsin</u>		
Tomahawk	L.S. Cheney	
	July 25, 1893	NY
Verona	H.C. Greene	
	74561	DAOM
 <u>Wyoming</u>		
Bear Lodge Mtns.	Griffiths & Carter	
	August 1897 (2 coll.)	NY
Sheridan	A. Nelson 10749	
	July 24, 1926	NY
Sundance Mtn.	A. Nelson	
	July 3, 1896	NY



Welcome

T.A. Williams

July 26, 1897

NY





## APPENDIX B

Herbarium collections of *Apiosporina morbosa* examined

## CANADA

Alberta

Edmonton	L.L. Kennedy	
	May 26, 1958	ALTA
Lac La Biche	W.J. Cody	
	39268	DAOM
Linnet Lake	L.L. Kennedy	
	August 12, 1961	ALTA
Seba Beach	I. Mounce	
	5134	DAOM

British Columbia

Agassiz	R.E. Fitzpatrick	
	19764	DAOM
New Westminster	E. Cooper	
	3043	DAOM

Manitoba

Rennie	W.G. Dore	
	91065	DAOM



Winnipeg	I.L. Conners	
	1637	DAOM
Winnipeg	I.L. Conners	
	1421	DAOM
<u>Newfoundland</u>		
Bay Bulls	A. English	
	36169	DAOM
Gander	L.J. Bassett	
	38752	DAOM
<u>Nova Scotia</u>		
Truro	A.R. Prince	
	July 4, 1925	ALTA
West Glenmont	I.L. Conners	
	3786	DAOM
<u>Ontario</u>		
Barry's Bay	F. Roll-Hansen	
	25912	DAOM
Chalk River	J.E. Bier	
	4703	DAOM
Charlton Sta.	F. Saunders	
	88947	DAOM
Gillies Lake	R.F. Cain	
	86103	DAOM



Hawkesbury	H.N. Racicot	
	3259	DAOM
Lake Temagami	R.F. Cain	
	86104	DAOM
Lake Temagami	I.L. Conners	
	1614	DAOM
Locust Hill	R. Baldwin	
	1400	DAOM
London	J. Dearness	
	36161	DAOM
London	J. Dearness	
	36166	DAOM
London	J.A. Parmelee	
	46051	DAOM
London	J. Dearness	
	36170	DAOM
Ottawa	J.A. Parmelee	
	26536	DAOM
Ottawa	I.L. Conners	
	2748	DAOM
Port Frank	J. Dearness	
	36163	DAOM
St. Catharines		
	36167	DAOM
St. Catharines		
	36168	DAOM





Quebec

Gatineau Park	R.G. Atkinson	
	18152	DAOM
Hull		
	36165	DAOM
Hull	Macoun	
	36171	DAOM
Laurentian Nat. Park	J.W. Groves	
	5282	DAOM
Rougemont	R. Crete	
	41161	DAOM
Ste. Blandine	L. Cinq-Mars	
	87681	DAOM
St. Pie	L. Cinq-Mars	
	41162	DAOM

Saskatchewan

Dana	I.L. Conners	
	551	DAOM
Dana	I.L. Conners	
	550	DAOM
Indian Head	P.M. Simmonds	
	1399	DAOM
Saskatoon	E.G.R. & R.C.R.	
	3646	DAOM



## UNITED STATES OF AMERICA

Colorado

Williams Canyon	K.S. Wilson	
	61716	DAOM

Idaho

Henderson		
	36172	DAOM

Iowa

Iowa City	L.L. Kennedy	
	May 12, 1955	ALTA

Kansas

Rockport	E. Bartholomew	
	36162	DAOM

Michigan

Leland	H.C. Greene	
	97138	DAOM

Wisconsin

Meadow Valley	H.C. Greene	
	67820	DAOM



## APPENDIX C

List of *Prunus* species examined with infections  
of *Apiosporina morbosa*

*Prunus armeniaca* L. var. *anda*

*Prunus americana* Marsh.

*Prunus melanocarpa* (A. Nels.) Rydb.

*Prunus nigra* Ait.

*Prunus pumila* L.

*Prunus serotina* Ehrh.

*Prunus spinosa* L.

*Prunus susquehanae* Willd.

*Prunus pennsylvanica* L.

*Prunus virginiana* L.

*Prunus virginiana* L. var. *melanocarpa* (A. Nels.) Sarg.

*Prunus* species











**B29858**